ANALYSIS OF ANION DISTRIBUTIONS IN THE DEVELOPING

STRATA OF A CONSTRUCTED WETLAND

USED FOR CHLORINATED ETHENE REMEDIATION

THESIS

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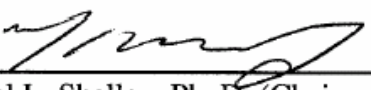
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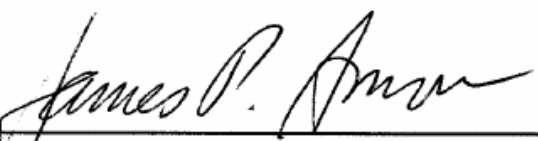USED FOR CHLORINATED ETHENE REMEDIATION

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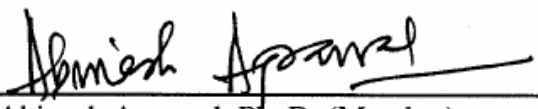
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Joshua D. Kovacic

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Abstract

Perchloroethene (PCE), Trichloroethene (TCE) and their degradation products are among the most common organic groundwater contaminants in the United States. Constructed wetlands utilizing upward flow harbor reduction-oxidation conditions that have demonstrated the potential to promote both partial and total mineralization of PCE and TCE through the process of natural attenuation.

Organic acid and inorganic anion concentrations are indicative of reduction-oxidation processes that drive chlorinated ethene degradation. These analytes were investigated to assess their development within three vertically stratified regions of a constructed wetland cell at Wright-Patterson Air Force Base fed by groundwater contaminated with PCE and TCE. Data collected during the months of January 2002, December 2002, and January 2003 revealed changes in the organic acid pool over time and in space that correlated with changes in the inorganic anion pool. Overall organic acid concentrations decreased by an average of 93% over 11 month period, indicating a substantial geochemical evolution of the organic acid pool over this timeframe. Measurements dissolved oxygen and ORP supported the existence of an aerobic region at the base of the wetland, followed by an anaerobic region in the strata above. Significant nitrate and sulfate reduction in the anaerobic region occurred in unison with the emergence of higher concentrations of lactate and formate. Results indicate the reducing conditions and substrates required to support reductive dechlorination of chlorinated ethenes were present in the subsurface of the wetland.

ANALYSIS OF ANION DISTRIBUTIONS IN THE DEVELOPING STRATA OF

A CONSTRUCTED WETLAND USED FOR CHLORINATED ETHENE

REMEDIATION

I. Introduction

Overview

The purpose of this study was to characterize a vertical profile of anion concentrations in an upward flow constructed wetland built for the purpose of remediating groundwater contaminated with Perchlorethene (PCE) and Trichlorethene (TCE). Analysis of this profile was used in conjunction with preceding research efforts to identify chemical processes that may shed light on the mechanisms of chlorinated ethene remediation in the hydric strata of an upward flow constructed wetland. Low-molecular weight, mono-carboxylic acids and inorganic anions were used to indicate biotic and abiotic processes occurring at different locations within the wetland. This study incorporated previously collected data as well as a 1-year follow-up comparison of these compounds to assess the developing subsurface environment within the wetland. Inferences based upon statistical analysis of selected analyte concentrations coupled with measurements of dissolved oxygen, oxidation-reduction potential, temperature, and pH placed emphasis on the role the analytes played in the identification of potential chlorinated ethene degradation pathways present in the wetlands.

Background

Chlorinated ethenes and their natural transformation products are the most common organic groundwater contaminants in the United States (McCarty, 1996). PCE is a volatile organic compound (VOC) and is among the three most frequently detected groundwater contaminants nationwide (National Research Council, 1997). Over the past three decades, the United States Air Force (USAF) and the Department of Defense (DoD) have identified thousands of sites containing groundwater contaminated with chlorinated ethenes such as PCE, TCE, isomers of Dichloroethene (DCE), and Vinyl Chloride (VC). PCE, TCE, and DCE are most commonly used as industrial cleaning solvents and degreasers. PCE is used in the dry-cleaning industry as well. Vinyl Chloride is primarily used in the production of polyvinyl chloride (PVC) plastic.

Chlorinated ethenes (primarily PCE and TCE) have been introduced into the groundwater as a result of a long history of careless usage and disposal practices, as well as through leakage from underground storage tanks and landfills. Classified as dense non-aqueous phase liquids (DNAPLs) because of their high density and relatively low solubility in water, chlorinated ethenes typically sink to the bottom of the aquifer where they are extremely hard to locate and remediate. As groundwater comes into contact with these DNAPL source areas, soluble constituents of the contaminant partition into the groundwater. The groundwater then carries them throughout the aquifer, allowing them to sorb and desorb to soil particles, creating a contaminant plume (Wiedemeier et al., 1997).

Chlorinated ethenes can have varying toxic effects on humans, depending on the compound. PCE and TCE are probable carcinogens that can cause liver and kidney

damage at high dosages (Masters, 1997). While 1,2-Dichloroethene is not a known carcinogen, it has been found to be associated with liver and kidney damage. VC, the most toxic of the chlorinated solvents, is a known human carcinogen. It can cause liver, kidney, heart, lung, and nerve damage, and can prevent blood from clotting [Agency For Toxic Substances Disease Registry (ATSDR), 2001]. TCE and VC are both listed in the ATSDR Top 20 Hazardous Substances (ASTDR, 2001). It is crucial that sites containing chlorinated ethenes be remediated to safe levels, especially where the potential exists for drinking water contamination.

The National Research Council classifies remediation of chlorinated solvents into three general categories: 1) technologies for solidification, stabilization, or containment of the contaminant; 2) technologies for separation, mobilization, and extraction of the contaminant from the subsurface; and 3) technologies for in-situ removal or transformation of the contaminant utilizing biological and/or chemical reactions (National Research Council, 1997). While the National Research Council estimated that pump-and-treat is used at approximately 89% of all contaminated groundwater sites, EPA data from 1995 data suggests that only 1% of groundwater cleanup sites utilize in-situ technologies.

The USAF currently uses the pump-and-treat method at the majority of its groundwater remediation sites. Although technologies such as these allow for a high degree of control during the remediation process, they are expensive as well as being energy- and maintenance-intensive. Consequently, cleanup cost for just one contaminated site can cost tens of millions of dollars (Masters, 1998). The Department of Defense alone has an estimated 7,300 sites contaminated with chlorinated aliphatic

compounds. The National Research Council (NRC) recently reported the cost of remediation at contaminated sites owned by Departments of Defense, Energy, Interior, and Agriculture and the National Aeronautics and Space Administration totals between $234 and $389 billion over the next 75 years (NRC, 1997).

Natural Attenuation

In the search for cheaper, more efficient remediation technologies, researchers have discovered that natural attenuation processes can be used to degrade contaminants into less harmful substances. Research indicates that natural microbial activity can degrade PCE into less chlorinated degradation products and, under the proper conditions, into innocuous end products such as carbon dioxide (CO_2) and water (Lee et al., 1998). This knowledge, coupled with new technologies in microbiology and the environmental sciences, has inspired researchers to investigate the microbial processes and interactions that lead to the bioremediation of chlorinated ethenes (Lorah and Olsen, 1999). One area of research focuses on utilizing constructed wetland environments to foster the necessary microbial activity needed to bioremediate groundwater contaminated with chlorinated ethenes.

Chlorinated aliphatic hydrocarbons may undergo biodegradation through three different pathways: energy-yielding oxidations, energy-yielding reductions, and co-metabolic pathways (oxidations and reductions) (Lee et al., 1998). When consumed in energy-yielding oxidations, less-oxidized chlorinated aliphatic hydrocarbons such as vinyl chloride and 1,2-dichloroethane act as an electron donor and can be used as a source of carbon, a primary energy source for cell growth and maintenance in the microbial population (Norris et al., 1994). Energy-yielding oxidations can occur in

aerobic or anaerobic regions.   When used in energy-yielding reductions, the chlorinated

hydrocarbon acts as an electron acceptor and not as a carbon source, and a chlorine atom

is exchanged for a hydrogen atom (Wiedemeier et al., 1997). This process only occurs in

the anaerobic region and is commonly referred to as halorespiration or reductive

dechlorination. Co-metabolic pathways utilize enzymes or cofactors that are fortuitously

produced during microbial metabolism to catalyze chlorinated hydrocarbon degradation

in both aerobic and anaerobic environments (Wiedemeier et al., 1997).

Aerobic Zones

Methanotropic Cometabolism

CO_2

Direct Oxidation

$O_2 + CH_4$ $+O_2$

TCE, cis DCE, VC

CH_4

Anaerobic Zones

Ethylene

Methanogenesis

VC

Organics, (H_2)

cis DCE

TCE

Reductive Dehalogenation

PCE
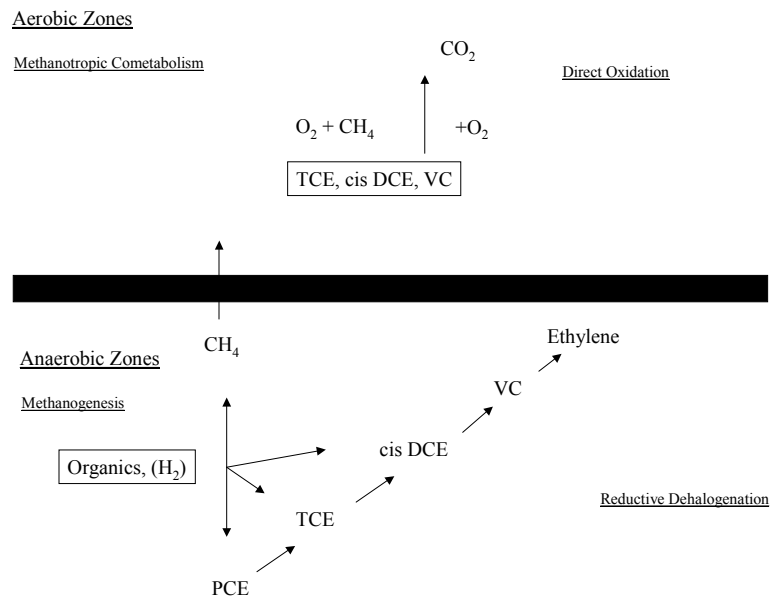
**Figure 1. Interplay between different biological mechanisms within a wetland
aquifer with both aerobic and anaerobic conditions. Chlorinated ethenes PCE,
TCE, DCE, and VC can mineralized to CH$_4$ and ethylene via methanogenesis
and reductive dehalogenation respectively in the anaerobic region, while TCE,
DCE, and VC can be mineralized via direct oxidation or cometabolism in the
aerobic region. (Opperman, 2002)**

The sequential breakdown products of PCE are TCE, DCE, VC and finally

ethene. Whereas all chlorinated ethenes can serve as electron acceptors in microbial

metabolism under highly reducing conditions, the affinity for degradation of each product

changes with the redox conditions of the region. Dechlorination of PCE and TCE to

DCE usually occurs under mild nitrate or Fe(III) reducing conditions (McCarty, 1997).

While the reduction of DCE to VC or the transformation of VC to ethene requires the

more strongly reducing conditions normally found in association with methanogenesis or

sulfate reduction. This sequence of reducing conditions does not occur naturally in most

terrestrial ecosystems. Thus, complete mineralization in a strictly anaerobic environment

difficult to obtain.   Mineralization of these less chlorinated products is more readily

achieved through direct oxidation in the aerobic region. Therefore, following a

sequential pathway through anaerobic followed by aerobic regions should provide for

more complete mineralization of the contaminant. Figure 1 illustrates this concept.

(Chapelle, 2001)

Constructed Wetlands

Constructed wetlands utilizing upward flow in theory provide an excellent

environment for fostering these processes. Contaminated groundwater can be pumped

through the hydric strata of the wetland, first encountering the anaerobic region where

PCE and TCE can be reduced, and then encountering the aerobic region where DCE and

VC can be oxidized. During the summer of 2000, the Air Force Institute of Technology

(AFIT) constructed two such wetland cells at Wright-Patterson Air Force Base in Dayton,

Ohio, to study the ability of constructed wetlands to degrade PCE naturally. The study

represents a joint effort between students and faculty of AFIT and Wright State

University. Both wetland cells are situated over an aquifer that is contaminated with a

plume of PCE. The site has been identified and documented with the Ohio

Environmental Protection Agency with no current requirement for remediation. The

concentration of PCE in the aquifer in the aqueous phase is approximately 0.05 mg/L. The cells represent what is hoped to someday develop into a low cost, low energy, pump and treat system that relies on natural processes to attenuate PCE. The concept is supported by findings at a U.S. Department of Defense site in Aberdeen, Maryland, where a natural, groundwater-fed wetland appears to demonstrate the complete destruction of trichloroethylene - a related VOC and also a degradation product of PCE (Lorah and Olsen, 1999).

Table 1. Composition of the Soil Layers (Entingh, 2002)

Layer	Cell 1	Cell 2
Top	Hydric Soil (likely root zone)	Hydric Soil (likely root zone)
Middle	Hydric Soil	Local, Iron-rich Fill
Bottom	Hydric Soil (organic matter added)	Hydric Soil

The soil composition of the subsurface media differed slightly in each cell. Both cells were constructed using three 18-inch soil layers. The first cell contained 3 layers of hydric soil (i.e., soil characterized by anaerobic or reducing conditions when saturated), the bottom layer of which consisted of a mixture of approximately 10% woodchips (from yard-waste supplied by the Greene County, Ohio Waste Recycling Program) added to the hydric soil to provide an initial source of available organic carbon to facilitate microbial growth. The second cell contained a middle layer of iron-rich soil sandwiched between two layers of hydric soil with no woodchips added. The purpose of the iron rich layer was to generate Fe^{+3} reducing conditions, which have been seen to promote the degradation of vinyl chloride (Bradley and Chapelle, 1997). The roots of wetland vegetation planted in the top layer of both cells permeated all three layers (Amon,

personal communication, 2003), providing oxygen to sustain different aerobic reactions in the root zone. These differences in media allowed for a comparison of soil compositions in order to make recommendations for an optimal design approach that would promote the most efficient contaminant removal. While the initial plan for this study included the investigation of both wetland cells, an error in the construction of cell #2 led to a compromise of the wetland's liner, precluding the use of this cell. Therefore, this effort focused on cell #1.

Early indications were that the Wright-Patterson Air Force Base wetland cell #1 effectively removed PCE from the contaminated groundwater. Little was known, however, about the specific processes that affected the contaminant's removal, where such processes are occurred, and their reaction rates. The ultimate goal of this and related research was to characterize these processes as they existed in the constructed wetlands. The results of this study add to efforts that seek to explore the potential of constructed wetlands to de-chlorinate PCE and other related substances, and will aid in the development of design guidelines should such a remediation approach prove relevant and successful.

Organic anions such as nitrate and sulfate that function as electron acceptors serve as indicators of redox conditions in an environment, providing valuable clues about the anaerobic or aerobic mineralization processes that may be occurring in different regions. Organic acids function both as by-products and substrates in the ecosystem. These acids can serve as an indicator of biological activity. Organic acids, especially acetate, are important products and substrates for diverse microbial processes, especially in anaerobic habitats (Seagren and Becker, 1999).

Previous research conducted by Bugg (2002) developed a preliminary characterization of the anion profile of chlorinated ethenes in cell 1. His thesis, "An Anion Characterization of a Constructed Wetland Used for Chlorinated Ethene Remediation," investigated 10 anion concentrations and hypothesized the roles that they may play with respect to microbial interactions and competition within the wetland strata. Although his research provided an initial characterization of the anions present within the developing strata of cell #1, additional detail from cell #1 was needed to better identify the microbial interactions and processes that were believed to be occurring as the cell matured. In its early stages of development, the subsurface processes in a constructed wetland are expected to be at a heightened level of transition. In addition to the disturbances caused by initial preparation and sampling during January 2002, successional processes were possibly the largest source of variance found within the wetland. The analysis comprised in this research effort sought to facilitate further a comparison of anion concentrations over time and space to answer questions about reported anomalies and to validate suggested inferences based on preliminary research. This information will be useful in analyzing the state of wetland dynamics. (Bugg, 2002)

Research Questions

This research focused on the following questions:

1. What low-molecular weight mono-carboxylic organic acids are currently prominent in each layer of wetland cell 1?

2. What inorganic anions are currently prominent in each layer of wetland cell 1?

3. Does comparative analysis of organic acid and inorganic anion concentrations in cell #1 one year after initial analysis reflect any significant deviation from initial findings?

4. Can this analysis provide quantifiable conclusions about the rate of development of desired microbial processes in constructed systems?

5. Can comparison of concentration levels of the selected analytes within each cell suggest enhancements in design, construction, and overall efficiency of constructed wetlands used for this purpose?

II. Literature Review

Research Principles

Research into the mechanisms that promote natural attenuation of chlorinated hydrocarbon contaminants in groundwater systems has become increasingly popular in recent years. These mechanisms, driven by the type and abundance of bacteria present, as well as the physiological processes they carry out, vary significantly from environment to environment (Chapelle, 2001). In the process of microbial metabolism, microorganisms remove electrons and transfer them to other chemicals to obtain energy from a substrate (Chapelle, 2001). This process is a vital element of natural attenuation and an important tool contributing to the classification of different subsurface environments. Understanding metabolism of natural attenuation first requires an understanding of the basic reduction and oxidation processes that drive biodegradation.

Oxidation-Reduction (Redox) Reactions

Reduction and oxidation reactions (collectively referred to as redox reactions) always occur in concert with one another. One compound is oxidized while the other is reduced. The compound that is reduced is referred to as the oxidizing agent. The compound that is oxidized is referred to as the reducing agent. Redox reactions can be separated into three general processes that essentially revolve around the transfer of electrons: 1) One compound is reduced, or loses oxygen atoms to another compound which gains oxygen atoms, or is oxidized; 2) One compound is oxidized, or loses hydrogen atoms to another compound that gains hydrogen atoms, or is reduced; 3) One compound is oxidized, or loses electrons to another compound that gains electrons, or is

reduced. In this case the compound losing electrons is commonly referred to as the electron donor and the compound gaining electrons is referred to as the electron acceptor. Oxidation-Reduction Potential (ORP) is a quantitative measurement of the electric potential for a molecule to be either oxidized (become an electron donor) or reduced (become an electron acceptor) while in the current region. It is not surprising that ORP trends tend to follow trends of dissolved oxygen in groundwater.

Biogeochemical Cycling

The incidences of different redox reactions correspond with different conditions present in the subsurface environment, and are essential to biogeochemical cycling. It is through such cycling that important chemical solutes such as oxygen, carbon, nitrogen, iron, and sulfur species move through microbial and botanical food chains, as well as through chemical transformations in the abiotic environment (Mitsch and Gosselink, 2000).

In environments where the absence of sunlight precludes photosynthetic production of oxygen, oxygen content is determined by the difference between rates of oxygen transport from the atmosphere and rates of respiration. The degree to which rates of oxygen transport into the system exceed or lag behind rates of respiration (oxygen leaving the system) will dictate whether the system becomes aerobic or anaerobic. Anaerobic conditions are more prevalent in aquatic sediments, because slow rates of water movement decrease the available flux of oxygen to these environments. (Chapelle 2001)

Under anaerobic conditions, organic carbon produced by plant photosynthesis can be partially cycled back to CO_2 through anaerobic oxidation. Fermentative

microorganisms partially oxidize the carbon to produce reduced compounds such as organic acids, alcohols, and molecular hydrogen (H_2). These compounds can then be more fully oxidized anaerobically using Fe(III), sulfate, or carbon as the electron acceptor. Under aerobic conditions, reduced carbon is largely cycled back to CO_2, with oxygen being reduced to water. (Chapelle, 2001)

Organic carbon can also provide energy for microorganisms that initiate nitrogen (N_2) fixation in the subsurface environment. N_2 fixation produces ammonia, which under aerobic conditions can be oxidized by nitrifying bacteria to nitrite, which is further oxidized to nitrate. Nitrate can then be assimilated and reduced to NH_2, and used as a component in protein synthesis by a variety of plants and microorganisms. The nitrate ion can also be used as an electron acceptor under anaerobic conditions. This reaction is thermodynamically favored for bacterial metabolism. Different microorganisms catalyze this type of nitrate reduction as it occurs over a series of steps; $NO_3 \rightarrow NO_2 \rightarrow N_2O \rightarrow N_2$, commonly referred to as denitrification. (Chapelle, 2001) Carbon supply appears to limit nitrate removal efficiencies where denitrification is the dominant mechanism of net nitrate removal, because microbes need the energy and reducing power gained from the oxidation of carbon compounds to carry out denitrification. Nitrate removal efficiencies are dictated by the C:N ratio, which can be manipulated by varying either carbon loading or the hydraulic loading rate of nitrate-contaminated water. (Ingersoll and Baker, 1998)

Following the consumption of dissolved oxygen and nitrate in the subsurface environment, ferric iron (Fe(III)) has the next highest affinity for use as an electron acceptor by a variety of microorganisms. Fe(III) is reduced to ferrous iron (Fe (II)), which remains sediment-bound, sequestered in iron-rich clays such as illite. Fe(III)-

reduction in the anaerobic environment remobilizes phosphate which is often adsorbed by Fe(III) oxyhydroxides in the aerobic environment. Phosphate and Fe(II) can then diffuse back into the aerobic zone. Once transported to an aerobic environment, Fe(II) can be re-oxidized to Fe(III) oxyhydroxides. (Chapelle, 2001)

Ferric iron reduction takes precedence over active sulfate reduction, which is next in the order of affinity for microbial metabolism. This process can occur in either an assimilatory or dissimilatory manner. Assimilatory sulfate reduction refers to the microbial reduction of sulfate to sulfide in order to provide sulfur for protein synthesis. Dissimilatory sulfate reduction refers to the use of sulfate as the terminal electron acceptor, oxidizing organic carbon and elemental hydrogen to produce hydrogen sulfide purely in the anaerobic region. (Chapelle, 2001)

Biodegradation Pathways of Chlorinated Ethenes

Biodegradation of chlorinated ethenes can occur through four primary degradation pathways: energy-yielding oxidations, co-metabolic oxidations, energy-yielding reductions, and co-metabolic reductive dehalogenation (Lee et al., 1998). Energy-yielding oxidations occur in either the aerobic or anaerobic region when certain microbial populations utilize chlorinated ethenes as a primary energy source for cell growth and maintenance.

Under aerobic conditions, the tendency of chlorinated ethenes to undergo oxidation increases as the number of chlorines present decreases (Chapelle, 2001). Therefore, VC has the greatest tendency for oxidation as a sole carbon substrate. Efficient microbial consumption of 1,2-DCE indicates that 1,2-DCE can also be utilized as a primary substrate to support microbial metabolism under aerobic conditions (Bradley

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and Chapelle, 2000). Bed-sediment microorganisms indigenous to a creek where DCE-contaminated groundwater continuously discharges, have demonstrated rapid mineralization of DCE and VC under aerobic conditions. Rates of DCE and VC mineralization increased significantly with increasing contaminant concentration, indicating beneficial value to microbial metabolism (Bradley and Chapelle, 2000).

Under anaerobic conditions, VC and more rarely DCE can oxidize to carbon dioxide under Fe(III)-reducing, sulfate-reducing and methanogenic conditions. VC can also be directly oxidized by NO_3-reducing and Mn(IV)-reducing bacteria (Chapelle, 2001; Bradley and Chapelle, 1996). Under methanogenic conditions, fermentative microorganisms such as acetogenic bacteria have been shown to transform VC to acetate, which is then oxidized to CO_2 by other terminal electron accepting processes such as Fe(III) reduction (Chapelle, 2001). However, the possibility remains that VC can also be directly oxidized by respiration.

Co-metabolic oxidations are an aerobic degradation process in which enzymes produced by a specific microbial process act upon a separate substrate to fortuitously oxide it. The ability of methanotrophic microorganisms to oxidize chlorinated ethenes to CO_2 is an example of such a process (Lee et al., 1998). While the occurrence of co-metabolic degradation is rare in groundwater systems due to the rare occurrence of significant concentrations of methane and dissolved oxygen existing together, conditions can be artificially created to support such processes.

Co-Metabolic reductive dehalogenation occurs in the anaerobic region as well. In this process, chlorine atoms in the molecular structure of the chlorinated ethene are replaced by molecular hydrogen. The sequential reduction of chlorinated ethenes

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ultimately yields ethene, which is environmentally safe. Reductive dechlorination of PCE and TCE to DCE isomers can be co-metabolically perpetuated by a variety of anaerobic microorganisms, including methanogens, acetogens, sulfate-reducing bacteria, and novel bacteria types that do not fall into either category (Bagley and Gossett, 1990).

Reductive dechlorination can be energy-yielding as well. Because chloroethenes are relatively oxidized due to the chlorine atoms they possess, they can also act as electron acceptors or oxidants in microbial metabolism (Chapelle, 2001). Recent studies have confirmed the existence of direct dechlorinators—microorganisms derived from contaminated subsurface environments and treatment systems—that utilize chlorinated ethenes as electron acceptors in energy conserving, growth coupled metabolism, referred to as dehalorespiration. The tendency for chlorinated ethenes to undergo reductive dechlorination decreases as they become more reduced (Chapelle, 2001). Therefore, the more reduced the molecule becomes, the greater the reducing conditions that are required to reduce it further. With the exception of oxygen itself, PCE is a stronger oxidant than most naturally occurring electron-accepting species. Thus, in the absence of oxygen, it will readily undergo reductive dechlorination to TCE. TCE can be reductively dechlorinated to cis-DCE under Fe(III) reducing conditions, followed by the reduction of cis-DCE to yield VC, which requires more strongly reducing conditions such as sulfate or reduction methanogenesis (Vogel et al., 1987). The lack of such conditions results in the relatively slow, incomplete transformations of chloroethenes observed at many field sites (Gossett and Zinder, 1997), which presents a problem when other degradation pathways are not favorable.

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Reducing Environment

While there is evidence for anaerobic oxidation of VC under Fe(III) reducing conditions (Lovely and Phillips, 1986), and the organisms that can mediate such processes are ubiquitous in the subsurface environment, mineralization of chlorinated ethenes in the anaerobic zone is not adequate to support a successful natural attenuation strategy (Gossett and Zinder, 1997). The increasing tendency for chlorinated ethenes to undergo aerobic oxidation as they reach their most reduced form provides rationale for the introduction of the chlorinated ethene to conditions supporting oxidation once it is relatively reduced (Chapelle, 2001).

It has been seen that the aerobic and anaerobic processes of chlorinated ethene degradation are highly dependent upon the reducing conditions present in the aquifer (Chapelle, 2001). Competition for electron donors such as hydrogen between dehalogenators is an important factor affecting dehalogenation in anaerobic environments (Chapelle, 2001). Hydrogen is an important substrate for many anaerobic bacteria, particularly Fe(III) reducers, sulfate reducers, and methanogens, in addition to reductive dechlorinators (Chapelle, 2001; Weijma et al., 2002; Smatlak et al. 1996). Each of these respirative processes has a different affinity for the consumption of H_2. CO_2 reduction has the lowest affinity, and steady state H_2 concentrations in methanogenic aquifers are therefore relatively high. Sulfate reduction has a slightly greater affinity for H_2, characterized by slightly lower concentrations of H_2 and Fe(III). Nitrate reductions have even higher affinity for H_2 and are characterized by progressively lower steady state H_2 concentrations. (Chapelle, 2001)

Consequently, when nitrate concentrations are sufficiently low, iron (FeIII) reducing conditions prevail, followed by sulfate-reducing and methanogenic conditions respectively (Vogel et al., 1987). Finally, when nitrate, sulfate, iron, and carbon dioxide are either non-existent or remain only in very low concentrations, halorespirators can successfully compete for electron donors to be used in metabolic processes. Conditions are now optimal for the degradation of chlorinated ethenes. This supports observations that reductive dechlorination tends to be more efficient under methanogenic and sulfate-reducing conditions than those of Fe(III)- and nitrate-reducing conditions (Chapelle, 2001; Bagley and Gossett, 1990). It has been shown that reductive dechlorination is driven by the amount of available molecular hydrogen present in the anaerobic environment (Tandol et al., 1994; Smatlak et al., 1996). This means that to a large extent, the success or failure of natural attenuation will rely upon the type of reducing conditions as well as the specific type of dechlorinator and amount of electron donors present (Gossett and Zinder, 1997).

Fermentation

Through the process of fermentation, organic electron donors appear to serve mainly as primary precursors to supply the hydrogen required for dehalogenation (Yang and McCarty, 1998). Whereas respiration refers to microbial metabolism utilizing external inorganic chemicals as electron acceptors, fermentation refers to microbial metabolism utilizing organic chemicals within the metabolic reaction as electron acceptors. It is primarily through fermentation that terminal electron-accepting processes can drive the supply of H_2 for dehalogenation.

Low molecular weight organic acids are frequently reported in oxygen-restricted environments because they are the result of the microbial decomposition of natural organic matter by fermentation (Cozzarelli et al., 1994). The variety of organic compounds contained in aquatic sediments offers a wide menu of potential substrates for microbial metabolism. This leads to a variety of species that can thrive on these different compounds and consequently produce different fermentation products. The resulting fermentation products become the basis for additional food chains. (Chapelle, 2001).

Hydrogen-producing fermentations begin with the production of pyruvate through the process of glycolysis. Pyruvate then goes through a multi-step process by which acetyl-phosphate and hydrogen may be formed (Chapelle, 2001; Varadarajan and Miller, 1999). Pyruvate can then be partially oxidized to a number of organic compounds with the production of additional energy (ATP) for the microbes. Among these organic compounds, acetate, lactate, formate, propionate, and butyrate are common end products of bacterial fermentation. Formate, lactate, and particularly acetate are important in ground-water geochemistry because they can also be used as electron donors by respirative bacteria (Chapelle, 2001; McCarty, 1997); however, the potential for such metabolic interactions varies greatly among different organic acids and communities of organisms (White et al., 1990). Figure 2 provides a basic diagram of the electron flow that stems from fermentation.

In aquifer microcosms, the electron donors used for the reductive transformations are most likely organic compounds present on aquifer solids or storage materials in microorganisms (Liang and Gribic-Galic, 1992).
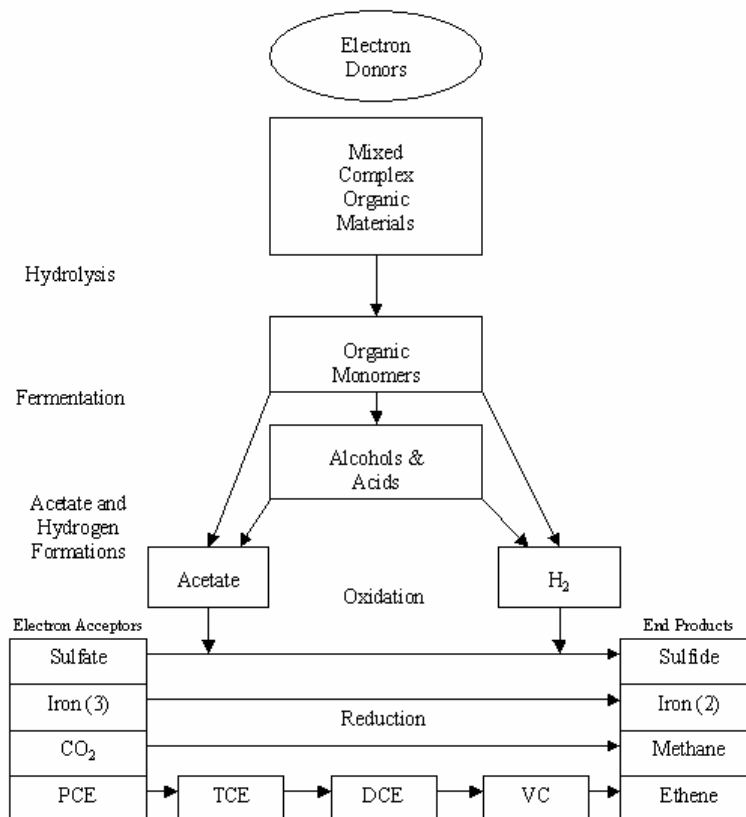
Figure 2. Electron flow from electron donors to electron acceptors in the anaerobic oxidation of mixed complex organic materials. Microorganisms that can use chlorinated compounds (PCE, TCE, cis-DCE, and VC) as electron acceptors in halorespiration compete for the electrons in the acetate and hydrogen intermediates with microorganisms that can use sulfate, iron (III), and CO_2 as electron acceptors. (McCarty, 1997)

A wide variety of microorganisms are able to couple oxidation of organic substrates with the reduction of nitrate in order to obtain energy for growth (Chapelle, 2001). Sulfate-reducing bacteria can be dependent upon fermentative bacteria to supply them with formate, acetate, and lactate, in addition to hydrogen. Of these, the oxidation of lactate to acetate with the liberation of hydrogen is the most well known pathway (Chapelle, 2001), and lactate has been seen to be the most effective source of carbon in field studies (Kleikemper et al., 2002). The fermentation of propionate in the absence of

lactate has been shown to be even more efficient at reductive dechlorination, with the ability to degrade TCE completely (Martin, 2001). In subsurface environments lacking other inorganic electron acceptors, methanogenesis, by either CO_2 reduction or acetate oxidation, is the predominant respirative process (Chapelle, 2001).

In anaerobic systems, the geochemical evolution of the low-molecular weight organic acid pools is controlled, in part, by the variety of electron acceptors available for microbially mediated electron-transfer reactions. The accumulation of aliphatic organic acids in anoxic groundwater can be attributed to the depletion of electron acceptors in aquifers (Cozzarelli et al., 1994). These conditions can be characterized by the concentrations of certain organic acids and reduced inorganic anions (Chapelle, 1995).

Identifying the distribution of microbially mediated redox processes is the key to predicting the fate and transport of organic and inorganic contaminants in groundwater systems. As the number of terminal electron acceptors becomes reduced, increasing concentrations of aliphatic acids such as acetate are observed. In anaerobic environments, complex organic compounds such as chlorinated ethenes can only be mineralized through the combined behavior of multiple, interdependent populations. Consequently, in these systems, continuous removal of reaction products by syntrophic populations is necessary to sustain the oxidation of these complex organic compounds.

In terms of contaminant bioprocessing capacity, methanogenic consortia appear to be more versatile than anaerobic systems using oxidized sulfur as a terminal electron acceptor (White et al., 1990). Studies with enrichment cultures of PCE and TCE degrading microorganisms provide evidence that, under methanogenic conditions, mixed cultures are able to completely dechlorinate PCE and TCE to ethylene (Freedman and

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Gossett, 1989). In these studies, it was found methanol was the most effective electron

donor to sustain reductive dechlorination of PCE and TCE, although hydrogen, formate,

acetate, and glucose were also sufficient.

Bagley and Gosset (1990) found that PCE was reductively dechlorinated to TCE

and *cis*-DCE by laboratory sulfate-reducing enrichment cultures. Neither lactate, acetate,

methanol, isobytyric acid, valeric acid, isovaleric acid, hexanoic acid, succinic acid, nor

hydrogen appeared directly to support PCE dechlorination, although lactate-fed cultures

demonstrated longer-term dechlorinating capability.

Anaerobic oxidation of VC and DCE to CO_2 under humic acid-reducing

conditions has also been demonstrated. It was shown that three times more ethene was

produced from dehalogenation of *cis*-DCE using propionate than benzoate as electron

donor. Subsequent reduction steps from *cis*-DCE to VC and ethene are much slower, and

often require a greater availability of electron donors to drive the reactions. In addition to

ethene, ethane, and CO_2, CH_4 can be a significant product of chloroethene biodegradation

in some methanogenic sediments (Bradley and Chapelle, 1999).

Microbial Acclimation Factors

The microbiota in the aquatic terrestrial environments are primarily found

attached to surfaces in the soils and sediments.   Microbes have the capacity for an

extremely rapid response to selective conditions of the environment. These microbes

have the most rapid biomass doubling times in the soils and sediments with favorable

conditions, and yet they have extraordinary metabolic mechanisms for survival in

compromising conditions as well. (White et al., 1990)

While it is not yet clear to what extent organic acids in an aquatic system reflect their natural or anthropogenic sources, what time scales are involved in their transformations, and which processes in the aquatic environment affect their properties the most, it is known that microbial metabolic processes do affect the nature and composition of the organic acid pool. These processes collectively result in an "aging process" in which the composition and properties of the organic acid pool change with time. (White et al., 1990).

Factors affecting Microbial Populations

The primary changes that microbial populations most often face include temperature changes, pH changes and changes in availability of new substrate. These are all interrelated to some extent. Temperature dictates microbial metabolism rates and completely halts metabolism in extreme cold. Conversely, as temperature increases, metabolism increases until reaching an optimum temperature level or "cardinal temperature" that is characteristic of a particular microbial strain. While the effect of temperature on metabolism also depends on pH and salinity, most subsurface bacteria operate most efficiently at a temperature range of 20-30 degrees C. (Chapelle, 2001). A recent study found that while temperature changes within this range did not affect the growth rate of sulfate-reducing bacteria, the bacteria's affinity for reducing sulfate increased with the temperature (Moosa et al., 2001). In addition to temperature, pH affects microbial metabolism by dictating the aqueous solubility and chemical complexing ability of groundwater. Low pH levels result in an increase of lipid solubility that decreases the bioavailability of organic acid substrates in ground water. (White et al., 1990). The rapid depletion of oxygen following the sudden influx of carbon

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substrates from contamination to an aerobic environment can lead to anaerobic conditions and anaerobic metabolism mechanisms involving the new electron donor substrates and new electron acceptors to go along with them. These new reducing conditions can lead to further microbial acclimation outcomes.

The extent to which the implications of microbial acclimation can be utilized to enhance bioremediation technology is uncertain. While evidence has reflected that significant microbial acclimation occurs in time periods of less than a year, other factors, such as electron-acceptor availability, often prove to be the more limiting factor to biodegradation. Thus, while the acclimation response certainly affects degradation of organic compounds in groundwater environments, further research is needed to support its application to bioremediation technology (Chapelle, 2001).

When conditions for microbial growth are substantially changed, such as the case with introduction of groundwater contaminants into the aquifer, microorganisms must adapt themselves to the new conditions. When the contaminant offers a potential substrate to support the microorganisms, the microorganisms must adjust, depending on how different the contaminant is from the normal substrate. Microbial populations can adapt to new substrates by induction of specific enzymes not present before exposure. The composition of the population can also change to reflect an increase in the number of organisms able to metabolize newly available substrates (Chapelle, 2001). Through natural selection, assuming that the contaminant offers a significant substrate, microorganisms that can utilize the contaminant in the most efficient way will thrive. The composition of the microbial population will then change as it becomes more acclimated to the presence of the contaminant. Therefore, rates of biodegradation via

reductive dechlorination will be a partial function of the acclimation of indigenous microorganisms to the contaminated conditions. In turn, this implies that biodegradation rates will change over time as acclimation proceeds, with the potential for increasing substantially as microorganisms become more acclimated. (Chapelle 2001).

Wetland environments are favorable for complete reductive biodegradation of chlorinated VOC's because the abundant supply of natural organic carbon from decomposing plant matter provides an ample supply of electron donors to initiate fermentation. The distributions of redox-sensitive constituents are favorable for the initiation of reductive dechlorination of chlorinated VOCs present in wetland sediments, whereas biodegradation of PCE and TCE would not be expected to be a significant process under the generally aerobic conditions of the aquifer (Lorah and Olsen, 1999).

In the effort to characterize the type and strength of reducing conditions present in ground water environments (nitrate-reducing, sulfate-reducing, etc…), ion-exchange chromatography based on high-performance column packings offers a reliable method to routinely detect the organic acids and inorganic anion concentrations in groundwater samples. This method offers sufficient detection sensitivity and peak separation capacity to quantify significant concentrations of these analytes that will characterize the types of reducing condition present in groundwater flow (Chen, 1996). Once identified, the distribution of reducing conditions can be used to reveal all of the potential pathways of degradation that chlorinated ethenes may be following (Chapelle, 1995). In addition, environmental monitoring equipment such as the YSI *Sonde* system offer a means for quantification of dissolved oxygen, conductivity, pH, temperature, and ORP in

groundwater, all of which are important parameters for characterizing conditions

affecting natural attenuation in the subsurface environment (YSI, 2003).

III. Methodology

Overview

Representative water samples were extracted from the soil matrices in the different strata of a constructed wetland cell located at Wright-Patterson Air Force Base, Ohio. Following sample collection, preparation and analysis of these samples was conducted nearby at the AFIT environmental laboratory. Additional readings of temperature, pH, oxidation-reduction potential, and dissolved oxygen were obtained from wells penetrating the separate strata.

Constructed Wetland Specifications

Two separate wetland cells were completed in September of 2000. The design of these cells was uniquely geared towards the purpose of treating chlorinated solvent contaminants.   Each cell consisted of 3 separate soil lifts situated over gravel and sand layers.   In cell #1, an 18" mixed layer of 90% hydric soil and 10% compost (wood chips) was topped by two successive 18" layers of hydric soil without compost. In cell #2, a middle layer of iron-rich soil was sandwiched by two equally thick hydric soil layers. Each cell had an approximate 120' X 60' footprint. Both were individually isolated from the surrounding environment by an impermeable Geomembrane liner (30 mil Water Saver PVC) to allow for better process control. Both cells were situated above an aquifer containing a large PCE contaminant plume with an aqueous phase PCE concentration of approximately 0.05 mg/L. PCE contaminated groundwater from the aquifer was pumped from an adjacent well to supply lines designed to distribute the contaminated water evenly throughout the wetland.   The supply lines consisted of three parallel 3" PVC

pipes that were evenly perforated over the length of each pipe. These lines were situated in the gravel bed beneath the wetland and ran its full length. The water forced through these pipes gradually worked its way up to the surface, and then flowed across the wetland surface to exit weirs located at one end of each wetland cell (Entingh, 2002). Figure 3 shows an as-built cross-section of cell #1.
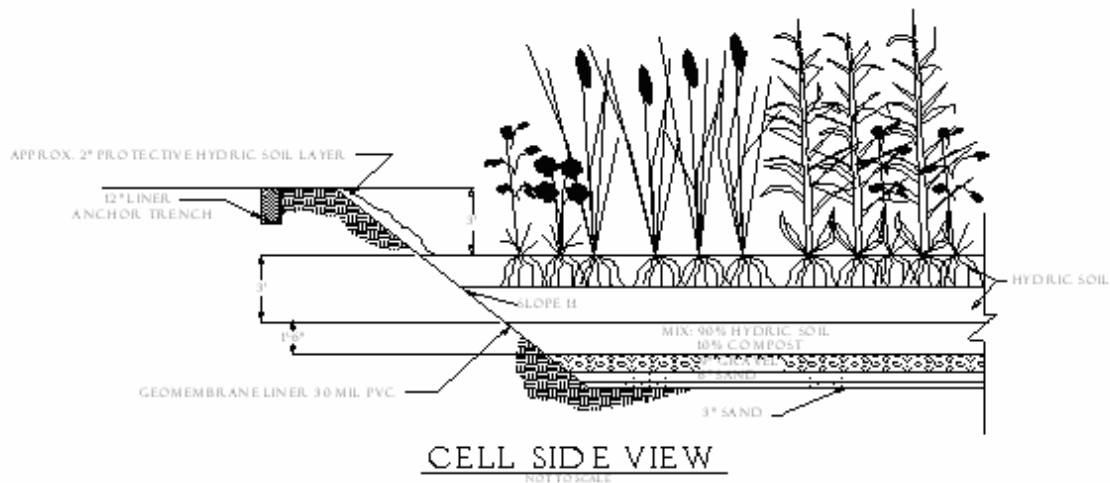
Figure 3. Constructed cell profile depicting three hydric strata, vegetation, and surrounding liner

Sampling Strategy

Previous researchers developed a sampling plan utilizing a stratified systematic placement of piezometers to accommodate four concurrent studies on the wetland. Included in this ongoing research effort were: 1) a study of the interactions of multiple facultative wetland plant species; 2) a characterization of hydraulic conductivity, piezometric head characterization, and coefficient of storage throughout the wetland; 3) a study of the remediation efficiency of chlorinated ethenes throughout the wetland; and 4) a study encompassing the initial research that is continued in this thesis effort.

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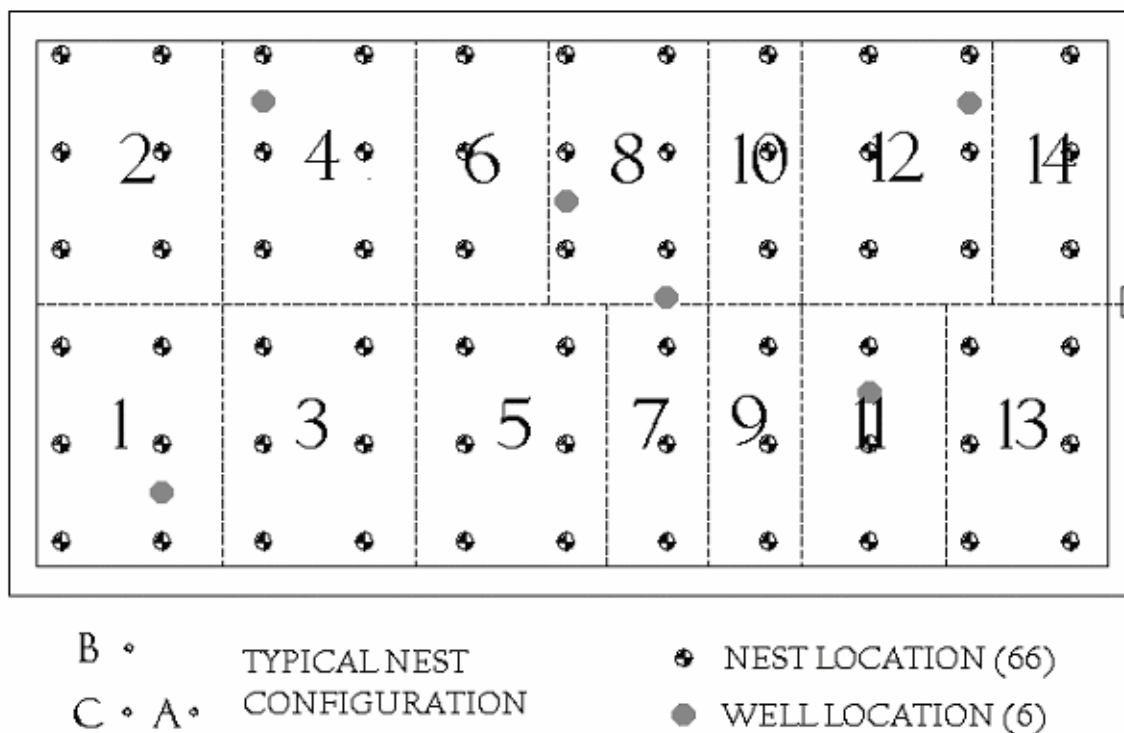
Figure 4. Vegetation subplots with associated piezometer and well nest locations

Table 2. Wetland Vegetation by subplot (Opperman, 2002)

Subplot	Piezometers included	Vegetation	Subplot	Piezometers included	Vegetation
1	1, 2, 3, 7, 8, 9	*Carex hystercina*	8	34, 35, 36, 40, 41, 42	*Juncus effusus Scirpus validus*
2	4, 5, 6, 10, 11, 12	*Scirpus atrovirens*	9	43, 44, 45	*Carex vulpinoidea*
3	13, 14, 15, 19, 20, 21	*Eleocharis erythropodo*	10	46, 47, 48	*Juncus torreyi Juncus dudleyi Carex lupiformis*
4	16, 17, 18, 22, 23, 24	*Carex comosa*	11	49, 50, 51	Mix
5	25, 26, 27, 31, 32, 33	*Acorus calamus*	12	52, 53, 54, 58, 59, 60	Blank
6	28, 29, 30	*Scirpus atrovirens*	13	55, 56, 57, 61, 62, 63	*Carex hystercina Mimulus ringens Penthorum sedoides Asclepias incarnata*
7	37, 38, 39	*Eleocharis acicularis Carex cristatella*	14	64, 65, 66	*Carex cristatella Carex vulipinoidea Penthorum sedoides Mimulus ringens Asclepias incarnata*

The cell was divided into 14 plots (including one unplanted plot) consisting of 17 different wetland plant species, as depicted in Figure 4 and listed in Table 2. Following their initial planting (Fall 2000) the plot boundary lines became less defined as the dominant species overtook several of the less competitive species. In addition, the plant root structures in the underlying strata became more developed, and are believed to have penetrated the full depth of the wetland. This differs from previous assumptions that the plant root structures would merely penetrate the top layer of the wetland (Bugg, 2002).

Researchers systematically placed 66 nests, each consisting of 3 piezometers penetrating the 3 individual stratum depths (198 piezometers total) over a 6 x 11 grid within the wetland. Each nest was configured in a rectangular footprint, with 1' distance separating the piezometers at the ends of the triangle from the piezometer at the center. Figure 4 provides locations of all piezometer nests as well as the configuration of the separate piezometer depths within each nest. Figure 5 provides a cross-section view of piezometer placement. A Solinst model 6150 piezometer was chosen as the means necessary for water extraction and peizometric head measurement. Water representative of the surrounding soil matrix entered the piezometer through a 6-inch screened area, the center of which was placed at the center of each respective stratum, as shown in Figure 5. Refer to Bugg, 2002, for further specifications on piezometers, piezometer installation, recovery, and development.

In October, 2002, six additional nests of wells were installed in both wetland cells to obtain conductivity, temperature, pH, dissolved oxygen, and oxidation-reduction-potential data using YSI Sonde monitoring equipment. These wells were comprised of 2.25" internal diameter PVC pipes with a 6-inch screened area consisting of alternating

1/16" slits every 1/8" on three sides of the pipe. The well nests were installed in the same

configuration as the piezometer nests, separated by approximately 13.5" on center, and

were supported by a triangular steel channel connecting the wells approximately 1' above

the wetland surface. The wells were installed using a method that continuously grouted

the hole with bentonite chips as the wells were simultaneously drilled and the pipe

installed. The number of well nests was limited to six, to minimize the number of holes

that would potentially allow water from the bottom layer to bypass the subsurface media

by flowing directly to the surface. The locations of the wells were picked arbitrarily to

provide for the most comprehensive reflection of the subsurface properties given the

limited number of well locations. The placement of these wells is illustrated in Figure 4.

Figure 5 illustrates a cross-section of the different well depths.
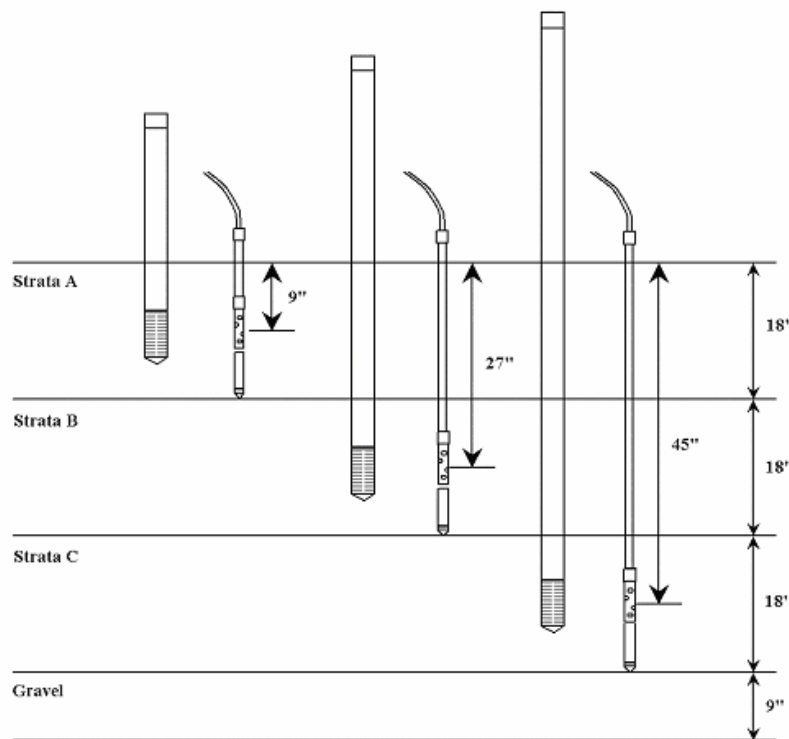
**Figure 5. Peizometer & well cross-sections showing the placement of screened area
at the center of each stratum**

The use of permanent sampling units such as the piezometer grid and well nests described here provided for more powerful statistical means of detecting change. This translated into a reduction in the number of samples required to detect a certain magnitude of change (Elzinga et al., 1998). The use of a permanent sampling grid also facilitated research to estimate long-term trends, define seasonal cycles, or forecast pollution concentrations (Gilbert, 1987, ; Mac Berthouex and Brown, 2002).

The systematic piezometer placement method and corresponding sampling plan were chosen to meet specific sampling objectives in the most efficient and realistic fashion. The primary sampling objectives in this study were to obtain an adequate amount of representative samples to provide an accurate characterization of the analytes of interest. This characterization would be used to make statistical inferences about the temporal changes within the wetland media and between individual strata.

Based on the assumption that all three strata are homogeneous throughout, the wetland was broken into three target populations per analyte corresponding to the three individual layers. The target population was considered to be the amount of target analyte found within the representative stratum. This population was considered homogenous throughout each stratum. Each 40mL sample was a subset of the population which, by comparison, could be considered infinite. Because natural populations have been seen to exhibit clumped spatial distribution patterns, random samples falling close together tend to correlate with one another, possibly leading to inaccurate conclusions about these populations. Therefore, using a sampling plan with units spaced far enough apart to reduce this correlation will tend to furnish a better mean and smaller standard error. Using the systematic grid placement method allowed for the assumption that the

samples were independent and could be considered random for statistical purposes (Elzinga et al., 1998). Disadvantages to the systematic plan included missing unsuspected anomalies over time and/or space due to patterns in wetlands that fit an offset pattern not consistent with the chosen grid, causing misleading estimates of the population mean and total (Gilbert, 1987). Three separate samples were to be taken from each sampling point over a one-month period to minimize periodicities and enhance the power of statistical analysis. However, due to the time and weather constraints, this number was limited to two full sampling passes from each layer. Samples used in this research effort included individual results from two complete sampling passes (198 total samples per pass; 396 total) during two two-week periods in December 2002. An average taken from three separate sampling passes collected by Bugg (2002) during January 2002 was also used. Individual sample values for samples collected in December 2002 are listed in Appendices AR-AZ. Sample collection dates are listed in Appendix BC.

Sample Extraction

Prior to extraction, piezometers were purged using 1/4" outer diameter, 1/8" inner diameter Teflon tubing connected to a peristaltic pump. Purging was necessary to obtain a representative sample that was void of stagnant water. While piezometers in the upper two strata could be purged completely, flow in stratum C was such that piezometers in this stratum could not be purged completely. In this case, 3 well-casing volumes were extracted from each piezometer in accordance with United States Environmental Protection Agency (EPA) recommendations (U.S. EPA, 1992). Water was purged from the top of the piezometric head, to ensure full removal of all stagnant water by means of

vertical flow. Piezometers were developed during the previous research effort to achieve

a minimum recharge rate of 3.0mL/hr in order to provide at least 70mL for sample

extraction following a complete purge twenty-four hours prior (Bugg, 2002). In most

cases the recharge rate was adequate to provide sufficient sample volume within several

hours.
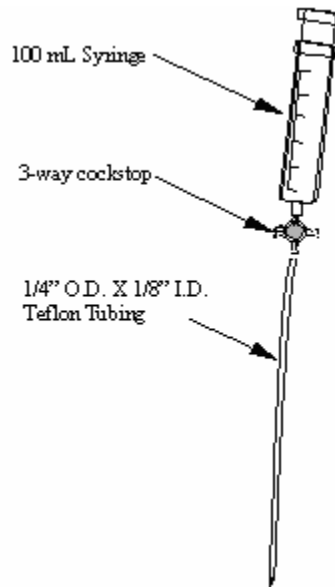
Figure 6. Sample extraction assembly (Bugg, 2002)

The syringe-tube assembly shown in Figure 6 was chosen as the extraction

instrument for its ability to provide minimal sorption and offer fine-tuned control of

purge and sampling rates (Bugg, 2002). It consisted of a 100mL glass jumbo syringe and

¼" Teflon tubing connected by a three-way cock-stop. A 50mL glass jumbo syringe

occasionally substituted the 100mL glass jumbo syringe. Following two flushes of the

extraction assembly with de-ionized water, the ¼" Teflon tube was inserted into the 1/2"

Teflon piezometer tubing until breeching the screened area. The first 20mL volume

extracted from the piezometer was discarded through the cock-stop valve. This served

two purposes - it prevented any residual de-ionized water from diluting the sample, while

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also priming the line to prevent air from coming into contact with the sample, thus minimizing the potential for analyte volatilization. After that, 50mL was extracted from the piezometer, 40mL of which was used to fill a 40mL glass vial. All vials were filled to the brim, creating a fluid meniscus at the top to prevent the encapsulation of any air bubbles within the vial when capped. See Appendix A for complete piezometer sampling procedures.

Sample Preparation Method

Following extraction, samples were taken immediately to the AFIT environmental laboratory for preparation and analysis. Samples were maintained in an ice bath while en route to the laboratory, and refrigerated at $3°$ C while awaiting preparation. Preparation required filtering the samples to reduce interference from other water soluble organic compounds that sometimes co-elute with organic acids to yield system peaks that interfere with organic acid detection (Chen, 1996). In addition, removing larger particles increased the life of the analytical column used for detection. To aid in ease of filtration, the 40 mL vials were first placed in a cooled centrifuge rotating at 800 rpm for 5 minutes immediately prior to filtration. This removed most of the larger particles from the sample, leaving only the smallest particles to be removed through filtration. Using a 10mL glass syringe, water was extracted from the 40mL vial and filtered into a Dionex disposable glass 2mL autosampler vial using a disposable 0.20 μm syringe Teflon membrane filter. Filters were used once and disposed of to eliminate any possibility of cross-contamination between samples. Between samples, the glass syringe was flushed twice with de-ionized water followed by one flush with the respective sample to be filtered. Samples were usually filtered and placed in the autosampler on the same day of

extraction. However, due to limitations on the rate of analysis, same-day analysis of all samples was not always possible. As reported by Bugg (2002), a laboratory study showed organic acids disappeared using a non-sterile organic-rich sample in 28 days (Kramer, 1990). To minimize degradation of the target analytes, total time from sampling to extraction therefore never exceeded one week.

Cleaning of the 40mL extraction vials between usages was a thorough four-part process. First, vials were rinsed in a dishwasher, and then submerged in a 1% *Liquinox* solution for twenty-four hours after which any residual material was scrubbed away using a test tube brush. The vials were then run through the dishwasher again using *LabSolutions* non-foaming powder detergent, and finally rinsed by hand in de-ionized water. Analysis of de-ionized water collected in vials cleaned using this method indicated no contamination effects on analysis results (Tara Storage, unpublished data). The equipment and procedure used for sample preparation can be seen in Appendices B and F.

Sample Analysis

Sample analysis was accomplished using ion exchange chromatography as the method for qualitative and quantitative detection of the organic acids and inorganic anions targeted in this study. Analysis was performed using Dionex PeakNet 6.30 software to operate a Dionex 600 series Ion Chromatograph (IC) system consisting of an EG40 Potassium Hydroxide Eluent Generator, GP50 Gradient Pump, AS50 Autosampler, CD25 Conductivity Detector, 4mm self-regenerating Ultra Suppressor (used in recycling mode), and a thermal compartment housing an AG-11 guard column and AS-11HC

separating column. Refer to Bugg, 2002, and the Dionex Manual for details on equipment specifications and ion chromatography principles.

Software Programming

The software method utilized in this research effort was modified from Bugg (2002), utilizing a continuous eluent concentration gradient to obtain identifiable separation of mono-carboxylic acid peaks. This modification was identical to the method used by Bugg, with the exception of a truncation of the final eluent gradient. Instead of ramping from 30 to 60mM KOH between minutes 28 and 38 of the analysis cycle, KOH was ramped from 30mM to 45mM between minutes 28 to 33. This allowed 5 minutes to be trimmed from the analysis time, increasing the rate of sample analysis and decreasing the wait time for samples awaiting analysis in the autosampler. While phosphate proved to be the last analyte to elute at 27.550 minutes, there were no analytes appearing in any of the samples after this time frame that would necessitate the extended eluent gradient increase used in Bugg's method. Appendix D shows the program used by the Dionex PeakNet 6.30 software to control the major components and parameters of those components during analysis.

The data resulting from the sampling and analysis methods outlined in the previous chapter is presented here to provide a detailed characterization of the target analyte populations as they exist in the different strata of the wetland. Analyte concentrations were obtained using Dionex Peaknet 6.30. Measurements of pH, oxidation-reduction potential, dissolved oxygen, and temperature were reported using YSI Ecowatch software. The data was manipulated using JMP IN v. 4.04, Paladise @Risk v. 4.5, and Microsoft Excel software for the purposes of statistical analysis. The

data was also manipulated using Surfer 8.01 software to provide contoured images reflecting patterns within wetland footprint. In accordance with the research questions posed in Chapter 1, this analysis provided: 1) a statistical interpretation of the data distributions unique to each analyte within the individual strata; 2) comparative tests to determine significant differences between strata, as well as significant differences between data sets collected during January 2002, and those collected during December 2002-January 2003; and 3) qualitative interpretation of trends and processes evident in the different strata.

Standards & Calibration

Combined liquid standards comprising the following analytes (listed in order of peak table appearance) were prepared manually by means of dilution from aqueous form or dissolved from a solid salt: Fluoride, Lactate, Acetate, Propionate, Formate, and Butyrate, Chloride, Nitrite, Nitrate, Carbonate, Sulfate, and Phosphate. Stock solutions (10,000 mg/L) stock solutions were prepared for each analyte with the exception of fluoride, which was already available in aqueous form at 1000 mg/L. Using data from January 2002, standard concentrations were combined in such a way that successive dilutions produced proportions representative of the expected ranges of all analytes. PeakNet 6.30 was used to create calibration curves using a multi-point linear calibration series.

Data from Bugg (2002), as well as Dionex literature provided qualitative analyte retention times used to identify peaks of interest on the chromatogram. Figure 8 demonstrates the typical peak characteristics for a combined standard. This particular standard did not include fluoride and bromide, which were mixed separately. Peaknet

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used the known concentration amounts input for the successive calibration standard dilutions to create calibration curves from area amounts automatically-integrated from the different chromatogram peaks. The software then used these calibration curves to calculate concentrations from chromatograms of unknown samples, reporting results from each analyte of a particular sample or sequence of samples in spreadsheet format. Calibration curves were created using ranges corresponding to the specific analytes as listed in Table 3. With the exception of carbonate, which was present even in blank samples of DI water, all calibration curves went through the origin. The correlation coefficient identified the degree to which the calibration curve correlated to the known calibration amounts that defined its slope. A minimum of 99.8% correlation coefficient was attained for all analytes.

Table 3. Calibration Table for External Standards

No.	Ret.Time (min)	Peak Name	Cal.Type	Points	Corr.Coeff. %	Offset	Slope
1	6.43	Fluoride	Lin	4	99.9964	0.00	245.10
2	7.08	Lactate	Lin	4	99.9998	0.00	32.09
3	7.49	Acetate	Lin	8	99.8394	0.00	43.2952
4	8.66	Propionate	Lin	4	99.9507	0.00	57.4182
5	9.59	Formate	Lin	6	99.9965	0.00	111.03
6	10.74	Butyrate	Lin	3	99.9973	0.00	25.7214
7	14.58	Chloride	Lin	6	99.9953	0.00	151.13
7	15.58	Nitrite	Lin	6	99.9980	0.00	108.86
8	19.17	Bromide	Lin	7	99.9972	0.00	69.64
9	19.61	Nitrate	Lin	7	99.9928	0.00	76.67
10	20.38	Carbonate	LOff	3	99.9735	119.05	12.07
11	21.83	Sulfate	Lin	6	99.9990	0.00	114.36

As reflected in Figure 7, the inorganic anions chloride, nitrite, nitrate, and sulfate had much greater peak resolution and separation compared to that of the organic acids lactate, acetate, propionate, formate, and butyrate. This caused several problems related

to the integration of the area under the peaks. At higher concentrations the peaks associated with fluoride, lactate, and acetate, respectively, tended to clump together, as evident in Figure 8. In addition, the peak associated with carbonate interfered with the peaks associated with nitrate and sulfate, as evident in Figure 9.
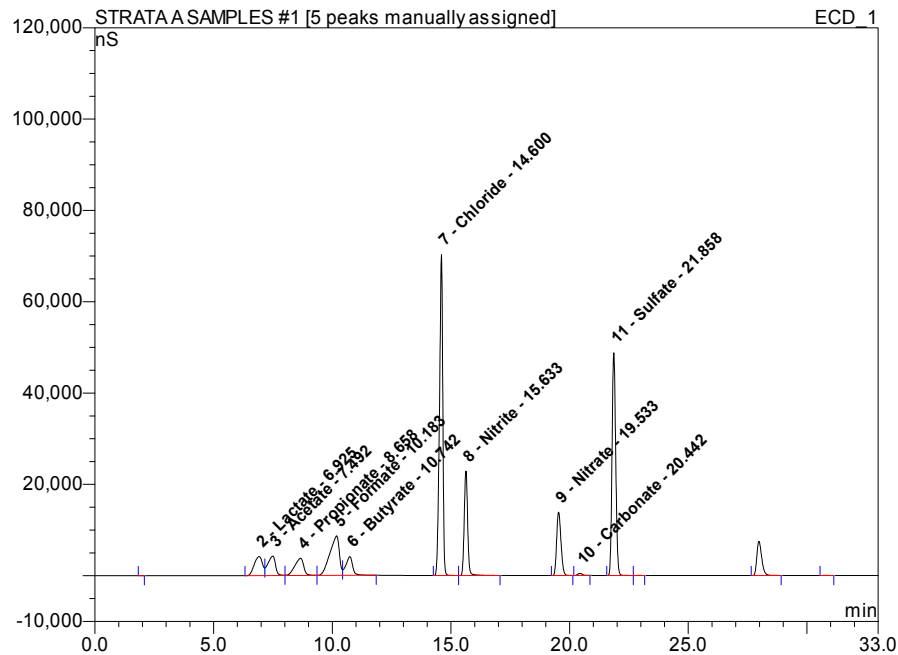
Figure 7. Typical mixed standard chromatogram, depicting peaks for lactate, acetate, propionate, formate, butyrate, chloride, nitrite, nitrate, carbonate, and sulfate (fluoride, bromide not included)
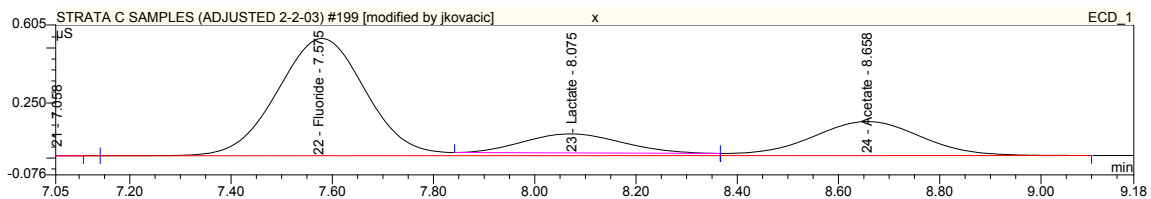
Figure 8 Fluoride, lactate, and acetate peaks in close vicinity
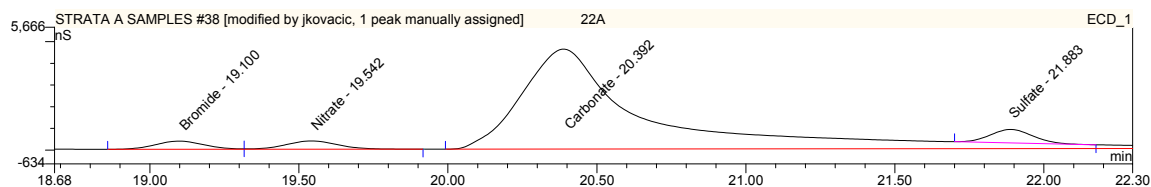
Figure 9. Bromide, Nitrate, Carbonate, and Sulfate peaks correctly integrated
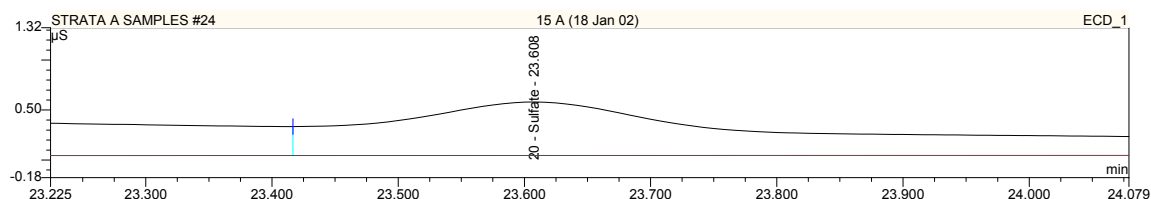
Figure 10. Sulfate incorrectly integrated on tail of carbonate peak, resulting in overestimation of sulfate concentration
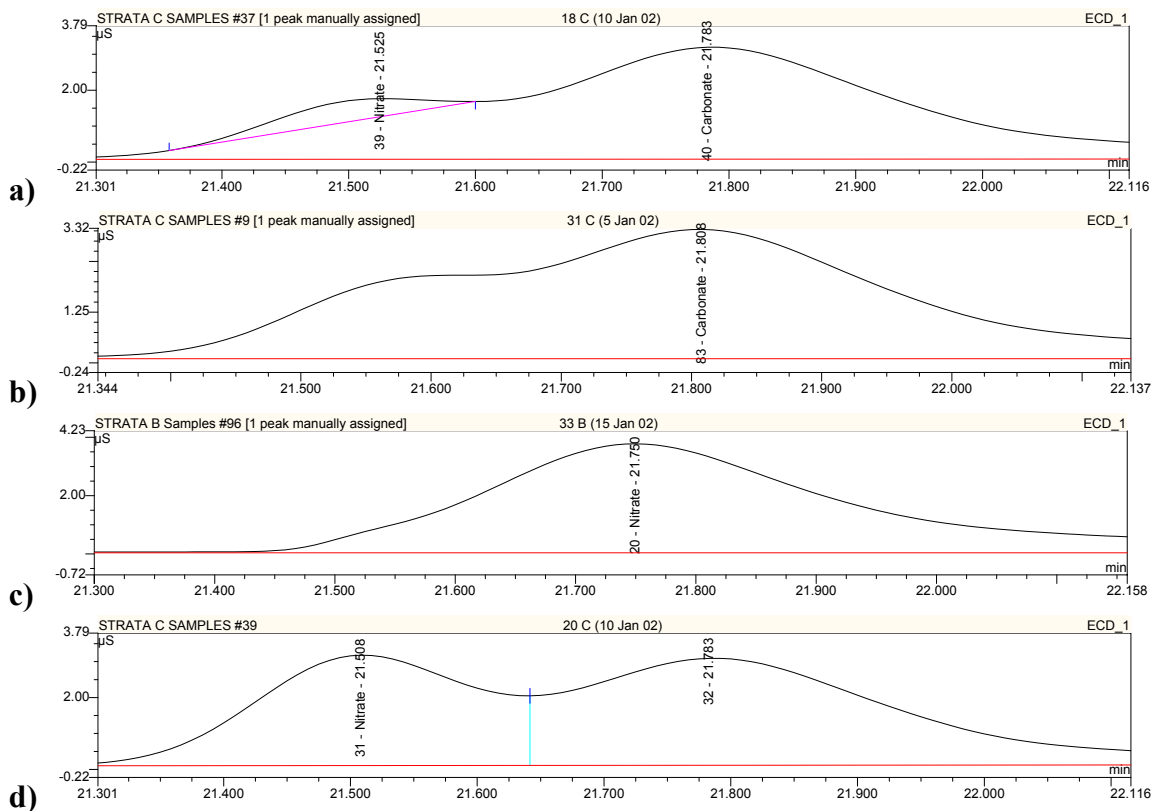
Figure 11. Nitrate incorrectly integrated resulting in underestimation (a), unrecognized resulting in underestimation (b), incorrectly identified resulting in overestimation (c) and correctly integrated (d) against Carbonate

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The auto-integration function in Peaknet was unable to handle this situation, causing it to incorrectly integrate interfering peaks as shown in Figures 10 and 11a-d, leading to inaccurate quantitative results. In these cases, individual peaks had to be manually integrated from the chromatogram associated with each unknown sample. In addition to this, chromatograms associated with samples taken in January 2002 (Bugg, 2002) were re-integrated manually to correct errors due to the inaccuracy of the auto-integration function initially used to report the data.

Method Detection Limits

The Code of Federal Regulations (CFR) defines the method detection limits (MDL) as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero (40 CFR 136, Chapter 1). Manufacturer specifications claimed MDLs for the equipment used in this study to be in the parts per trillion (ppt) range. Results from tests conducted to determine MDLs, according to procedures outlined in Part 136 of Title 40 CFR, supported this claim. Table 4 summarizes the MDL for each analyte, with a corresponding t-value of 3.143.

Table 4. Method Detection Limit for All Analytes [parts per trillion (ppt)]

Acetate	Butyrate	Formate	Lactate	Propionate	Bromide	Chloride	Fluoride	Nitrate	Nitrite	Sulfate
16.19	202.96	221.58	35.43	12.28	123.6	9.17	4.64	228.1	5.67	223.47

Background and Blanks

This study acknowledged two backgrounds of interest: 1) influent into the constructed wetland and 2) de-ionized (DI) water as the "instrument background." Table 5 provides a comparison of concentration averages of three separate influent analyses for the time periods over which the present study and the current study occurred. Table 6 provides a similar comparison of instrument background concentrations at the time of analysis. While the instrument background concentrations did not appear to vary significantly between years, there was significant distinction between influent concentrations; most notably, organic acids were absent, while nitrate and nitrite increased.

Table 5. Influent Concentrations

	Acetate (ppb)	Butyrate (ppb)	Formate (ppb)	Lactate (ppb)	Propionate (ppb)	Bromide (ppb)	Chloride (ppm)	Fluoride (ppb)	Nitrate (ppm)	Nitrite (ppb)	Sulfate (ppm)
Jan-02	0.9665	0.71719	7.5997	15.553	1.0386	N/D	78.717	158.89	3.4439	2.0224	50.729
Dec-02	N/D	N/D	N/D	N/D	N/D	25.167	67.419	171.83	10.78	13.7	40.361

Table 6. Instrument Background Concentrations

	Acetate (ppb)	Butyrate (ppb)	Formate (ppb)	Lactate (ppb)	Propionate (ppb)	Bromide (ppb)	Chloride (ppb)	Fluoride (ppb)	Nitrate (ppb)	Nitrite (ppb)	Sulfate (ppb)
Jan-02	0.38218	N/D	1.26535	1.73703	0.50440	N/D	6.05990	N/D	2.78141	N/D	380.324
Dec-02	N/D	N/D	0.21709	0.77192	N/D	N/D	7.83061	N/D	0.56932	N/D	328.401

Outliers

Outliers were identified according to the same method used by Bugg (2002). Based on the premise that the target population is considered to be the amount of a

specific target analyte that exists within the stratum from which the representative sample is taken, each target populations is represented by a data set consisting of one complete sampling pass (66 samples) through one strata of the wetland. Using these data sets a 95% confidence interval was obtained for each population. Average data from January 2002 was considered as one pass, due to the inability to distinguish between January 2002 complete sampling passes from data records. Data not located within a 95% confidence interval was labeled as an outlier. The following conditions were used to separate true anomalies from false data.

1.) Is the identified outlier similar to other quantitative measurements from the same piezometer?

2.) Are similar concentration levels found in the adjacent area?

3.) Does it support a trend in the population?

4.) Can the outlier be explained from unique phenomena or changes in sampling and analytical methods?

Several outliers from both studies were identified as erroneous due to instrument error and removed from the study completely. There were also many outliers that reflect speculative phenomena of bypass occurring within the piezometer nests. In these instances, water from stratum C is believed to be bypassing the top two layers, producing a false characterization of the strata at these locations. This occurred primarily around nests numbered 9, 10, 15, and 16. Outliers not meeting the 95% confidence interval are identified in Appendices AN-AW by a "*" preceding the concentration amount. With the exception of the false outliers that were removed completely, all outliers are represented in the contour maps in appendices A-AA to allow for a visual interpretation of possible

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trends and anomalous behavior. However, for purposes of gaining more representative

average amounts, all outliers were removed from the sample set when fitting the

distribution in order to find the best mean and standard deviation. Each data set was

evaluated using Paladise @Risk v. 4.5 (formerly BestFit), which identifies the best fitting

distribution and corresponding statistics for each population based on the Chi-Squared

distribution test method. Figure 12 shows an example of the software's curve-fitting

output. Distributions and corresponding statistics for each population are listed in

Appendix BB.
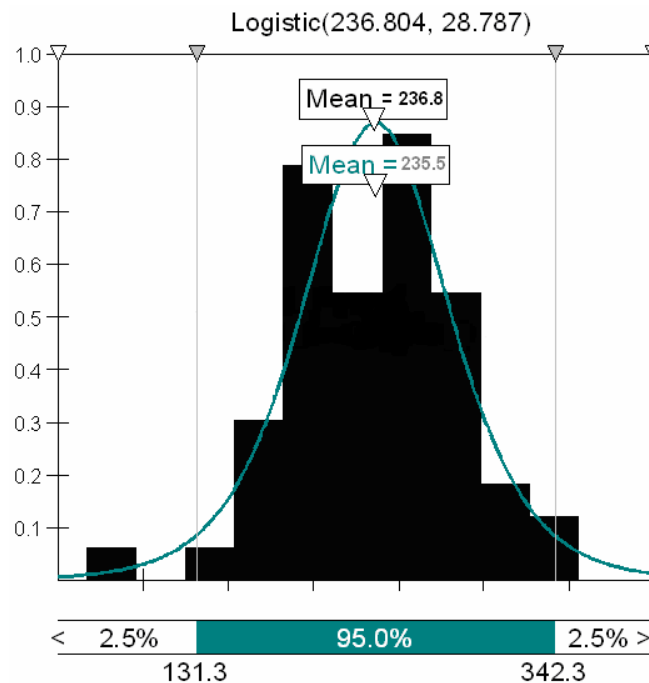
**Figure 12. Fluoride, Strata A, December 2002 (1) data with mean 235.53 ppb,
fit to a logistic distribution with mean 236.804 ppb and standard deviation 28.787.**

Figure 13. YSI 556 Multiprobe System (Sonde) (YSI, 2003)

Sonde Data Collection

Additional data was collected from the wells using YSI 556 Multiprobe System (shown in Figure 13) equipped with five sensors to simultaneously measure dissolved oxygen, pH, conductivity, temperature, and ORP. Using the sonde devices in the wells (a practice commonly referred to as "downhole" sampling) provided an accurate method for taking measurements of these parameters from water samples that were representative of the undisturbed water in the subsurface wetland soil matrix. Since the water chemistry of water stored in the well casing can be influenced by a number of factors, including sorption and leaching effects from the well-casing and screen materials, chemical changes due to the bentonite (clay) seals, and the presence of the air-water interface at the top of the water column, wells required purging to remove stagnant water prior to data collection. Using a method similar to that which is recognized by the EPA as "low-flow purging," fresh water within the well screen was hydraulically isolated from any stagnant water in the well casing, so the water sampled was taken from the screened interval only directly adjacent to the pump intake. This strategy was based on the concept that the

velocity of groundwater flowing through the well screen was sufficient to maintain an exchange with the adjacent formation water (YSI, 2003).

"Low-Flow" refers to the velocity of the water entering the well screen, which minimizes the disturbance to the surrounding groundwater system by minimizing the well drawdown (U.S. EPA, 2003). The shallow wells penetrating stratum A had a much lesser recharge-rate than the deeper wells and could be purged dry. Approximately three complete well casing volumes, at a rate of approximately 3mL/s could be purged from wells penetrating strata B and C without causing significant drawdown. Following purging, data collection could began promptly by lowering the sonde sensor device to the screened area of the well casing.   In cases of deeper wells, the sensor device was left to set for up to one hour until sonde detectors reflected that target properties had reached a steady state. At this point, data was recorded at 5-second intervals for approximately one minute. For the shallow wells, the Sonde sensor was lowered into the well only after the well had recharged enough to submerge the Sonde sensors. The Sonde was then left to equilibrate as described above. Detailed procedures used in this study for sonde data collection are listed in Appendix C. Data included in this study consisted of the results collected in 3 separate data collection passes that included the wells in each of the six well nests in cell #1 (18 per pass, 54 total) and were conducted on 23 December 2002, 8 January 2003, and 9 January 2003.

IV. Results & Discussion

Population Comparisons

Mean data as calculated using @Risk software was used to facilitate comparison
of populations by strata as well as over time. Figures 14a-c and 15a-c display the
differences between mean analyte concentrations found in inflow and outflow
measurements, as well as within the three strata. The graphs were separated according to
organic acids and inorganic acids, to aid in the interpretation of visible trends.
Concentrations were labeled for inflow, stratum C, stratum B, and stratum A respectively.
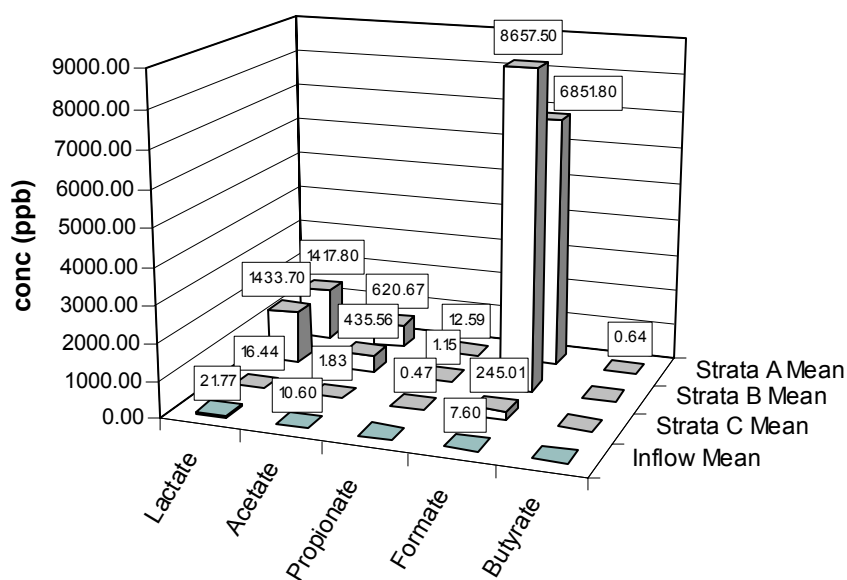
Figure 14a. Organic Acid Concentrations, January 2002

In samples taken from January 2002, it was apparent that formate was the most
prevalent organic acid, followed by lactate, and acetate, respectively. The sudden
presence of high concentrations of lactate, acetate, propionate, and most of all formate in
strata B and A following stratum C levels that are much closer to the inflow

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concentrations indicated a significant change in conditions between the center of stratum C and the center of stratum B. There were several possible explanations for this scenario. These possibilities are based on the potential presence of two driving forces that dictate organic acid levels in the subsurface environment: the production rate of organic acids through fermentation, and the rate of consumption of these organic acids as substrates in microbial metabolism. The first possibility was that organic acids were being produced in all strata by means of fermentation, but only the reducing bacteria in stratum C had an affinity for utilizing these organic acids substrates. Thus, the absence of organic acids in stratum C would be explained because they were all consumed. However, an increasing accumulation of organic acids would then be expected in the strata above, which did not occur. Instead, organic acid concentrations remained relatively stable between strata B and A. A more likely scenario is that the organic acids were being produced by the fermentation of organic materials starting somewhere between strata C and B. As they were carried upward, the microbial populations were unable to utilize them as fast as they were being produced, which explains their steady presence in the upper strata. The varying concentrations of the different organic acids indicated that the most prevalent type of microbial metabolism was primarily producing lactate, acetate, and formate. In addition, the relative levels of these three organic acids may provide inferences about the extent to which pyruvate is being partially oxidized. Lactate concentrations that were higher than acetate concentrations may indicate this cycle was being limited by the number of sulfate-reducing bacteria capable of oxidizing lactate to acetate. Consistently higher levels of formate, combined with lower levels of its oxidation product, lactate, may indicate that the microbial population was unable to utilize formate as a substrate.

Results from samples collected in December 2002 were significantly different. Organic acids detected in samples during this timeframe were found to contain much smaller concentrations of lactate and formate, with acetate, propionate, and butyrate being detected sparingly if at all. While the overall lack of organic acids detected in the samples from strata C were partially explained by the lack of organic acids detected in inflow samples from December 2002 as compared to those from January 2002, it was expected that the total amount of organic acids produced by fermentation would increase as plant life within the wetland matures, leading to the accumulation of decomposing organic matter within the wetland sediment. If this was the case, the likely explanation for the lack of organic acids that were so prevalent 11 months earlier is that microbial populations had become far more efficient at metabolizing them. The only other possible explanation was that fermentation was no longer producing these organic acids. However, this explanation is unlikely.

Following the same trend as samples taken the previous January, organic acid concentrations tended to be lowest in stratum C and highest in the stratum B, although concentrations of lactate and formate in strata A varied. In this timeframe, lactate concentrations in stratum B were on average between two and five times higher than those of formate.

This trend was opposite that seen during the previous winter, pointing to the possible conclusion that the composition of either the microbial populations producing these compounds, or that of the microbial populations able to consume these compounds, or both, had changed. Another possibility could be that the initial organic matter in

stratum C has been substantially depleted, limiting the fermentative production of organic

acids in this region.
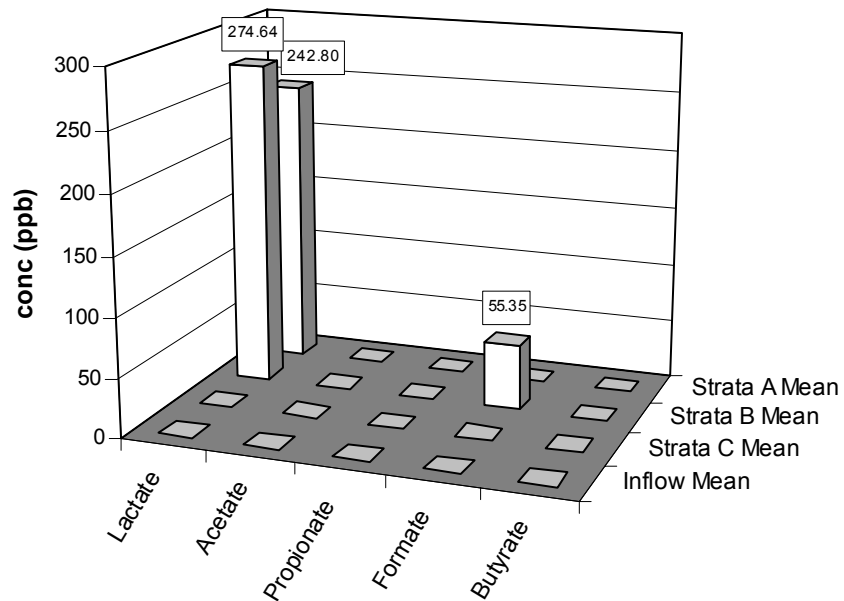
Figure 14b. Organic Acid Concentrations, December 2002 (1st Sampling Pass)
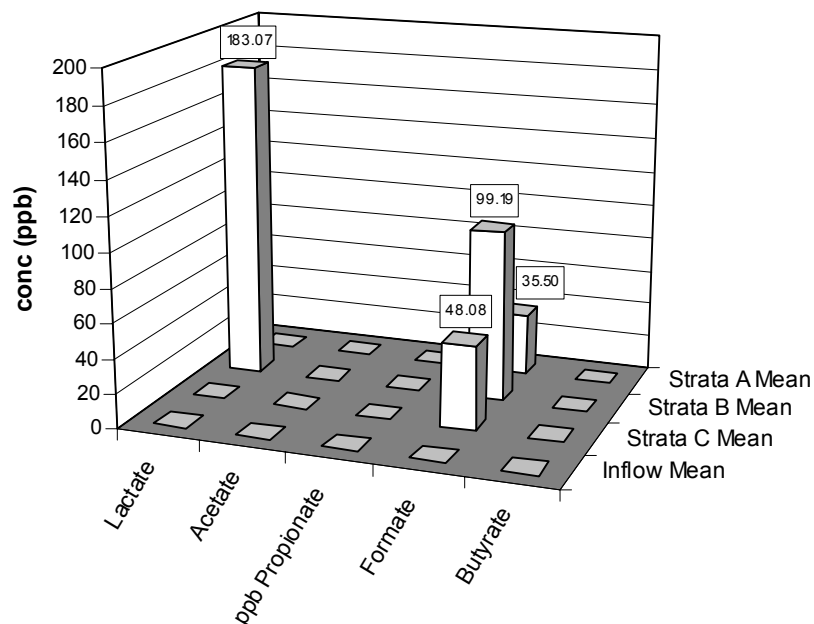
Figure 14c. Organic Acid Concentrations, December 2002 (2nd Sampling Pass)

A significant change in formate and lactate concentrations was also seen just within the December 2002 timeframe between the two two-week time periods during which the samples were taken. In the first December sampling pass, average lactate and formate concentrations rose from zero in stratum C to roughly 275 ppb (parts per billion) and 55 ppb respectively, in stratum B. In stratum A, average formate concentrations dropped back to zero in stratum A, while average lactate concentrations remained at 243 ppb. In the second December sampling pass average lactate concentrations rose sharply from zero to roughly 183ppb in stratum B, then dropped back to zero in stratum A. Formate concentrations during this pass rose to 48 ppb in stratum C, doubled in stratum B, then fell back to 35 ppb in stratum A. The relative increase and decrease in average formate concentrations between strata is roughly the same, indicating the presence of a factor affecting the baseline level of formate during this timeframe. The disparity between the higher concentrations of lactate in stratum B and its lack of persistence in stratum A from the first pass to the second pass indicated that some factor was influencing the microbial populations' affinity for producing lactate via fermentation in the region between strata C and B, and consuming lactate in the region between strata B and A. Other than time, the only external factor that differed between these two sampling phases was the weather. Both snow accumulation and subfreezing temperatures causing ice to accumulate on the wetland surface were prevalent during the second pass, while weather during the first sampling pass was much milder.

In the course of re-integrating data peaks from January 2002, several discoveries were made concerning the targeted inorganic anions. Peaks for nitrate and sulfate in strata C from January 2002 were incorrectly integrated. This caused a false reflection of

lower nitrate concentrations in strata C than in the other two strata when, in fact, manual

re-integration revealed nitrate levels in strata C to be higher than those in the other two

strata. The mis-integration of sulfate was somewhat erratic, but resulted in a general

over-estimation of sulfate concentrations present in all layers (see Figure 10).
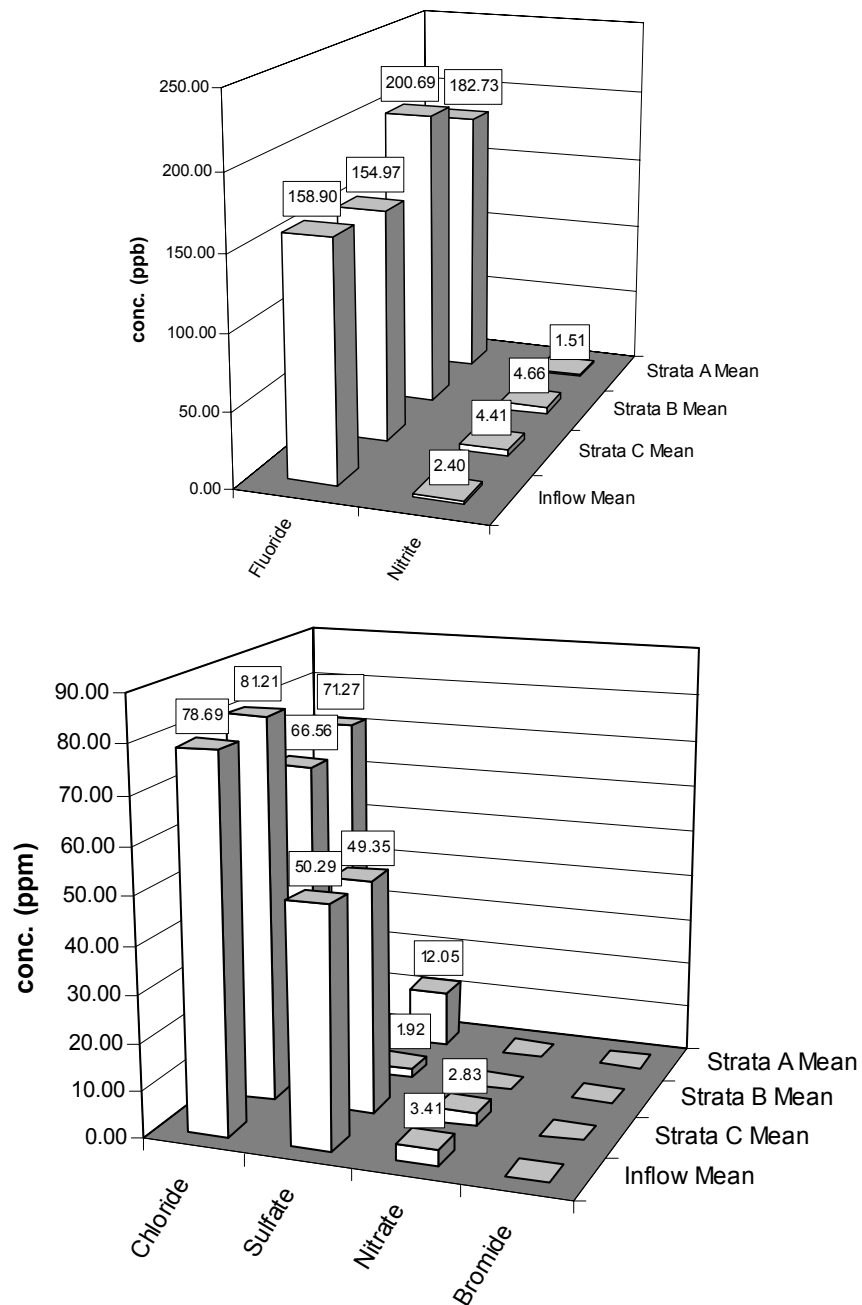
Figure 15a. Inorganic Anion Concentrations, January 2002

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In addition, a small unknown peak appeared slightly earlier than the nitrate peak in apparent trends within strata A and B. Dionex literature classifies peaks in this area to be associated with bromide, trifluoroacetate, or sorbate. Based upon standard test runs using dilutions of sodium bromide solution, this peak was believed to be associated with bromide. After-the-fact calibration curves were created and applied to the unknown sample chromatograms for all recent samples, as well as samples from January 2002 to provide at least some means of relative quantification of the presence of this analyte. Values for this calibration curve are also listed in Table 3.

Following this correction, inorganic anion concentrations in the three strata followed trends that were more comparable between January 2002 and December 2002. Nitrate and sulfate were both reduced to zero between strata C and A, although it appeared that most reduction occurred between strata C and B. Interestingly, inflow concentrations of nitrate in December 2002 were roughly three times greater than in those from January 2002. The cause of this substantial increase is unknown, although increases in nitrate concentrations are commonly attributed to anthropogenic sources such as the use of nitrogen-rich fertilizers.

Nitrite levels decreased rather consistently from strata C to B, but there were significant differences in its behavior from layers B to A over the separate sampling passes in December 2002. During the first pass in early December when average outdoor temperatures were warmer, nitrite levels continued to decrease between strata B and A. Later that month when the weather was much colder, nitrite was reduced to almost zero in stratum B, then jumped sharply in stratum A to levels more than twice the original levels in stratum C.
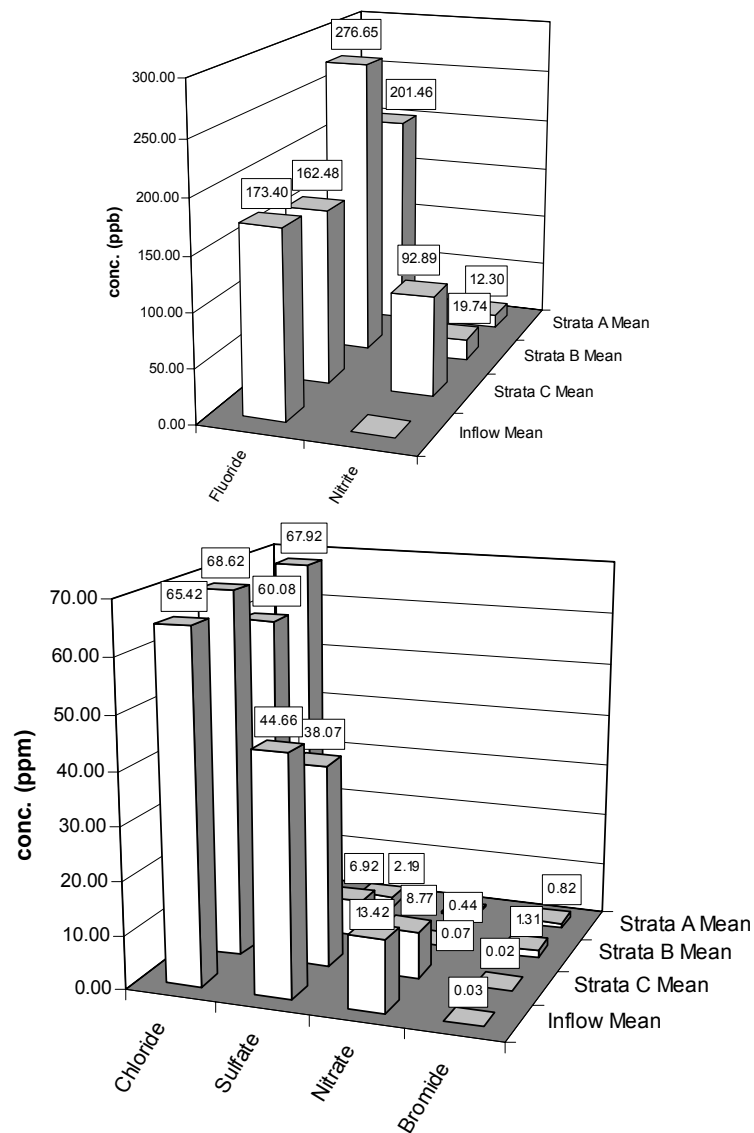
Figure 15b Inorganic Anion Concentrations, December 2002 (1)

Since nitrite is the product of reduced nitrate, and conversely nitrate is the product of oxidized nitrite, these collective observations offer details as to which conditions might have driven the nitrogen cycle within the wetland. The near-total reduction of nitrate in stratum C in every case was expected, due to its high affinity for hydrogen consumption. Upon visual inspection of the concentration contour plots of nitrite for all

strata, for all sampling passes, regions of high concentration were somewhat mottled

across the wetland footprint, separated by regions of low concentrations or none at all.
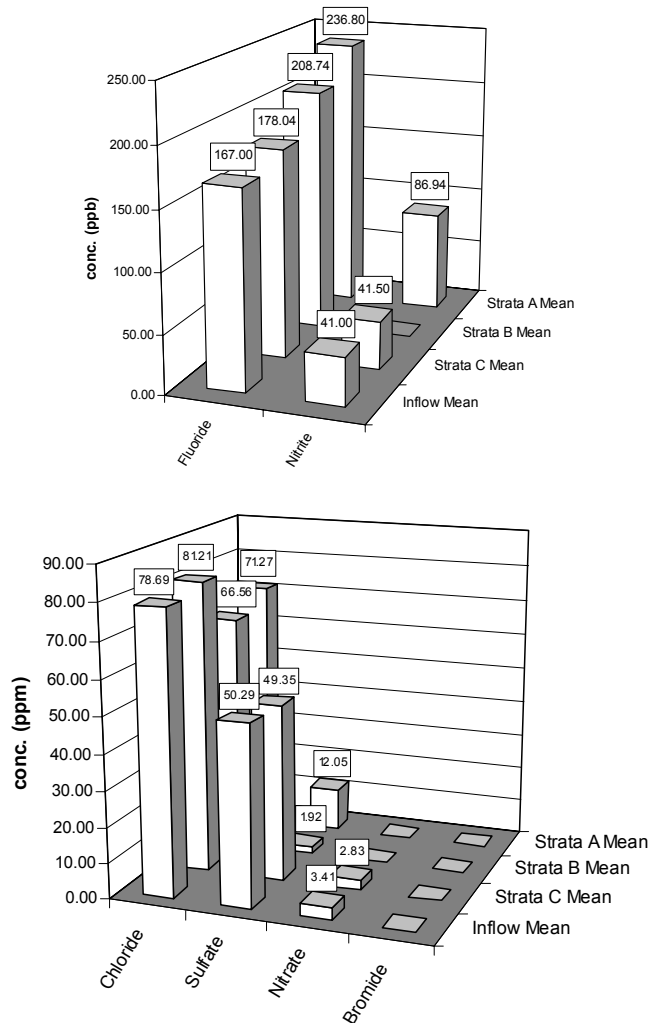
Figure 15c Inorganic Anion Concentrations, December 2002 (2)

This could indicate the presence of different reducing conditions or sub-regions in

which the microbial populations had a specific affinity for utilizing a particular nitrogen

substrate in redox reactions. While reducing conditions appeared to be promoting

complete reduction in some regions, reduction was apparently incomplete in other

regions. Comparison of the first and second sampling passes in December 2002 revealed

that nitrate reduction appeared to be more efficient between strata C and B towards the end of the month. The general absence of nitrate in the upper two strata indicated that the nitrite produced was not being oxidized back to nitrate while in the wetland subsurface.

Sulfate reduction appeared to be taking place following nitrate reduction. This was especially evident upon visual inspection of the sulfate concentration contour plots, where it could be seen that instances of higher sulfate concentrations in strata B and A seems to mimic concentrations of nitrate found in the same layers. In this case, it appears that the presence of nitrate was precluding sulfate reduction.

Fluoride remained relatively constant over all 3 populations, but the appearance of what was believed to be bromide in samples from December 2002 followed interesting trends, where previously in January 2002 much smaller concentrations had been detected. There appeared to be little to no bromide at all in stratum C in any of the sampled populations. However, the appearance of bromide in strata B and C indicated a difference in the subsurface conditions in these locations. While the appearance of bromide coincidentally corresponded with the reduction of sulfate and nitrate between strata C and B, there is no known connection between this compound and the microbial reduction of sulfate or nitrate. Possible factors affecting the detection of bromide include issues influencing its solubility in the groundwater. This may include the differing compositions of the wetland strata, the reducing conditions present within the strata, or other factors such as temperature, and pH. Chloride remained relatively constant, with a slight dip in concentration in stratum B. This research study also tried to identify the presence of phosphate, but no phosphate concentrations were found in any of the strata.

Although lacking comprehensive coverage because the limited number of monitoring wells in the wetland, contour plots of data collected on three occasions during the December 2002/January 2003 timeframe using the Sonde device provided a glance at the temperature, pH, ORP, and dissolved oxygen components of the subsurface environments within each strata. The water temperature varied between 6 and 12 °C. It was warmest upon entering strata C, and the temperature progressively dropped as it flowed upward. This trend was observed to be reversed in warm weather, when the water flowing into the wetland was significantly colder than outside air temperatures and became progressively warmer as it flowed upward. The measurement of pH was seen to hold relatively constant between 6.75 and 7.15, with a slightly decreasing trend evident from stratum C to stratum A. Dissolved oxygen concentrations ranged from 0-6 mg/L, but were highest in strata A and C. Heightened oxygen concentrations in stratum A were expected due to its proximity to the surface and the mass of the interacting root tissue. Similar oxygen concentrations in stratum C were attributed to dissolved oxygen already present in the inflow. Oxygen was depleted by redox reactions between strata C and B. ORP measurements showed that reducing conditions existed in all strata, however, they were significantly stronger in strata A and B than in strata C. Average ORP readings in strata A and B hovered around -100mV, while those in strata C were closer to zero. This supported the observation of sulfate and nitrate reduction in strata A and B.

Variability and Bias

Consideration of the sources of variability and bias that can exist in different aspects of a research study is vital to the validation of data results and inferences that are

based upon those results. These sources can be separated into variability and bias related to analytical analysis of the data, and to methods used in data collection and preparation.

In this study, the sources of variability and bias related to data collection and preparation were driven primarily by the potential of the target organic acid analytes to degrade and volatilize in relatively short amounts of time (MacGowan and Surdam, 1994). Because of this, the time between the purging of piezometers and collection of samples, as well as the time between sample collection and analysis was determined to be a source of variability or bias. However, multiple tests conducted on wetland samples purged, collected, and analyzed within the timeframes used in this study indicate that this source of bias was negligible. Speculation from the previous study that the sample-vial cleaning methods could be introducing a significant source of error was also discredited. In the effort to minimize volatilization of organic acids between sampling and analysis, methods were modified to eliminate headspace in both the 40mL sampling vial and 1.5mL autosampler vial. Additionally, glass autosampler vials were used in place of plastic autosampler vials to minimize any sorbing of organic acids to the container surface.

A more significant source of error may have been introduced during sample analysis. Instrumentation hardware replacements from one study to the next could have introduced some element of bias the data results from each study. As discussed earlier in this chapter, the close proximity of fluoride, acetate, and lactate peaks, as well as that of nitrate, sulfate, and carbonate peaks on the ion chromatogram, could have inhibited the ability of the ion chromatography software to automatically identify and quantify the area under each peak. Consequently, every sample had to be manually integrated. While

these peaks were integrated in the most consistent way possible, the placement of peak baselines and horizontal limits was somewhat subjective. The added element of human error into these quantifications introduced a source of variability and bias. This source of variability and bias is considered to be low, due to the consistent application of peak integration methods practiced by the technician, however, the precision of the concentration findings as they related to one another may be higher than the overall quantitative accuracy as a result of this method.

Finally the most significant source of variability and bias in this study may have been the weather. Bacterial metabolism can be extremely sensitive to changing temperatures. Weather conditions during January 2002 were much milder and temperatures much warmer than those in Decemeber 2002 and January 2003. While temperatures during both studies remained at levels much colder than the range under which microbial metabolism occurs most readily, these changes should be noted.

V. Conclusions and Recommendations

Synopsis

The purpose of this study was to characterize the concentrations of organic acids and inorganic anions that are indicative of the diverse distribution of geochemical processes and conditions occurring within the subsurface of an upward flow constructed wetland. Comparative analysis of these characterizations was used to demonstrate the existence of reducing conditions that potentially affect the chlorinated ethene degradation, the objective for which the wetland was built. As a continuation of the preceding study by Bugg (2002), this research effort focused on a comparison of data collected for that study during January 2002, with data collected during December 2002 and January 2003. Most recent data also included measurements of pH, temperature, dissolved oxygen, and ORP.

Based on the concept that hydrology, vegetation, and soil type in an upward flow constructed wetland would produce a scenario to carry chlorinated contaminants in the groundwater through consecutive anaerobic and aerobic subsurface regions, stronger reducing conditions were expected to be evident in the lower strata of the wetland. These conditions were expected to decrease progressively in the regions closer to the wetland surface, due to a closer proximity to the air and to higher rates of oxygen being infused into the subsurface by plant roots. As an indicator of these conditions, organic acids were expected to exist in the greatest concentrations within the lower strata of the wetland where conditions should support the fermentation of humic substances. In addition, the reduction of inorganic anions was expected within the lower strata as an indicator of the reducing conditions at these depths.

Findings from both studies indicated that the strongest reducing region did not occur below the center of the stratum C. On the contrary, the reducing region appeared to begin between the center of stratum C and stratum B, roughly 27 to 45 inches below the wetland surface, and was perpetuated in the upper strata of the wetland, to the center of stratum A, at a depth of 9 inches, which was the shallowest point from which samples were collected.

Evidence supporting the absence of reducing conditions at the center of stratum C included the overwhelming presence of nitrate and sulfate in this region, a lack of organic acids, neutral ORP measurements, and dissolved oxygen concentrations that were higher than the upper two strata. Evidence supporting the existence of reducing conditions in strata A and B included the sudden depletion of nitrate and sulfate in these strata, the increased presence of the organic acids formate, lactate, and acetate, high negative ORP measurements, and low dissolved oxygen concentrations. Due to errors found in the quantification of data in the previous study, these findings were in contrast to conclusions made in the earlier study which claimed that nitrate reduction was not occurring in the wetland.

The absence of organic acids from samples collected in December 2002 as compared to the much larger concentrations detected from samples in January 2002 indicated that the microbial processes within the wetland sediment had developed to a point such that the microbial populations either 1) no longer produced these organic acids as a product of fermentation, or 2) had become much more efficient at metabolizing these organic acids. Depletion of the original supply of organic matter in the stratum C could lend to the possibility that organic acids are not being produced as readily. The

implications of these alternatives on the degradation of chlorinated ethenes are vastly different, as the microbial populations that have been seen to promote reductive dehalogenation can indirectly utilize these organic acids as an electron donor.

As expected, the microbial processes supporting the conditions observed above did not occur in a homogeneous distribution within each stratum, but instead seemed to occur in a very heterogeneous array within each stratum across the entire footprint of the wetland. Visual inspection of analyte concentrations laid out on a contour plot provided much more detailed insight when deciphering the complex conditions and reactions that were actually existing within different 3-dimensional sub-regions of the wetland. While the possible sources of these distributions are virtually limitless, it is hoped that relationships between the organic acid and inorganic anion concentrations in these sub-regions can be combined with information from concurrent and future studies in the wetland to determine what specific reactions are taking place.

Recommendations

The primary limitation of the inferences that can be made based upon findings in this research effort is the timeframe under which it was conducted. All sampling efforts analyzed were conducted in December and January, when cold temperatures inhibited the rates of nutrient cycling and microbial metabolism, and wetland plant species were dormant (Mitch and Gosselink, 2000). Sampling at this time did not provide a representative picture of what was occurring in the wetland subsurface during the majority of the year, when warmer temperatures would enhance these processes. In addition, cold weather caused ice formations inside the piezometers, which made sampling difficult.

Further research is encouraged to facilitate a seasonal comparison of the target analytes in this study during the spring, summer and fall timeframes. In addition, the residence time of water flowing through the wetland was kept relatively constant during both studies, and the level used resulted in quicksand-like areas and areas of high bypass from one stratum to the next within the wetland. Research into the effects of increasing the residence time by lowering the flow-rate into the wetland may provide valuable information relating to the flow-rate parameters that support optimum microbial metabolism. It might also be of benefit to examine the possibilities of aerating portions of stratum A to sustain a more prominent aerobic region in which DCE and VC may be oxidized more efficiently (Bae et al., 1995).

Once cell #2 is repaired, a comparison between findings from cell #1 and cell #2 using current methodology could be used to evaluate the relative effect of the iron-rich layer in cell #2 on chlorinated ethene degradation. Correlation of the results from this study to those of concurrent studies to develop cation characterizations as well as characterizations of the chlorinated ethene breakdown products present in the wetland could be used to validate inferences made in this study and to better tie these inferences to the bioremediation of chlorinated ethenes. In addition to levels of pH, dissolved oxygen, temperature, and ORP discussed in this study, measures of other properties such as conductivity, dissolved organic carbon (DOC), CO_2, H_2, CH_4 emissions, and the presence of iron in its different oxidation states may better explain future observations and provide a greater degree of confidence to the inferences made based on these observations. The distribution of wetland plant species in cell #1 had evolved through successional processes into a less ordered layout than the original configuration. A

separate study utilizing more consistent gardening practices within the wetland could enable cell #1 to once again be stratified into vegetation sub-plots that could be correlated with the presence of different organic acids and reducing conditions.

With respect to the sampling method itself, an internal standard could also be added to samples that might decrease variability and improve the accuracy of analyte detection by accounting for the matrix effects (Bugg, 2002). Varying the sample plan to include focus on different areas of the wetland and changing sample sizes as well as the number of replicate samples taken per sample pass could add extra confidence levels to future research (Bugg, 2002).

Conclusion

The results from this study indicate that the reducing conditions and substrates required to support the reductive dehalogenation of chlorinated ethenes were present between the middle of the strata C and A. Sulfate-reducing conditions such as those observed in this region were seen to support the co-metabolic reductive dechlorination of PCE, TCE and DCE (Clemmer, 2003). While the presence of lactate and formate indicated the fermentative production of these potential organic acid substrates, there was insufficient evidence in this study to determine if, at what rate, and by what bacteria they were being consumed. Sampling techniques were not adequate to demonstrate the presence of an aerobic region in stratum A which would be desired to support the oxidation of less chlorinated products of reductive dehalogenation such as DCE and VC, however, results from Clemmer (2003) suggest that such conditions may be present. In order to deduce the effectiveness of the parameters used for the constructed wetland in this research effort, further research is required to determine the magnitude of the

reducing potential in subsurface regions in all strata, and to identify the presence of

aerobic conditions in the stratum A that will support complete mineralization of PCE and

TCE to ethene.

Appendix A: Piezometer Sample Extraction Procedure

1. Purge piezometers with peristaltic pump.
   NOTE: For strata A and B, piezometers could be fully purged by pumping from the bottom of the piezometer screen through ¼" Teflon tubing extension. Stratum C piezometers were purged by attaching the pump directly to the piezometer tubing and extracting three well casing volumes. Average time elapsed between purging and sampling of each stratum ranged between 0 and 24 hours, depending on weather conditions and rate of recharge.
2. Draw approximately 20 mL into the syringe, using the syringe assembly shown in Figure 6.
3. Turn valve on cockstop to open the purge valve while closing the tubing valve.
4. Purge the syringe contents.
5. Turn valve on cockstop to open the tubing valve while closing the purge valve.
   NOTE: Steps 3-5 serve two purposes: Rinsing any residual DI water from the syringe with water from the representative sample, while simultaneously priming the tubing assembly to minimize air bubbles drawn into the syringe with the sample.
6. Draw 50mL of piezometer sample in the syringe.
7. Turn valve on cockstop to open the purge valve while closing the tubing valve.
8. Purge 40mL of sample into a 40mL vial, creating a surface meniscus at the vial rim to minimize the entrapment of air bubbles in the sample when capped.
9. Cap the vial.
10. Turn cockstop valve to open tubing valve while closing the purge valve.
11. Insert Teflon tubing assembly into DI water reservoir.
12. Draw, at a minimum, 50mL of DI water into the syringe.
13. Gently shake syringe with DI water to keep all suspended particles in the DI solution.
14. Once 50mL have been drawn into the syringe, continue to pull back on the plunger while removing tubing assembly from DI water reservoir, so that air is drawn into the syringe. Pull the syringe plunger completely out of the syringe, letting the DI water flush out the back end.
15. Re-insert the plunger into the syringe, flushing air back through the ¼" tubing assembly.
16. Repeat steps 10-14.
17. Repeat steps 1 thru 22 to obtain samples each piezometer.
18. In between usage, rinse entire syringe assembly with DI water three times before and after use.

Appendix B: Sample Preparation Procedure

1. Pull 5 mL of a wetland sample (in the 40 mL vial) into a 5 mL glass syringe.
2. Discard the 5mL of wetland sample in appropriate reservoir.
3. Pull 5 mL glass syringe from same wetland sample.
4. Attach 0.20 µL syringe filter onto 5mL glass syringe.
5. Gently push the 5 mL sample from syringe through filter and into a 1.5 mL capacity autosampler vial.
6. Mark with corresponding number from wetland sampling point.
7. Flush 5 mL glass syringe twice with deionized water.
8. Discard remaining wetland sample from 40 mL vial into the appropriate reservoir.
9. Repeat steps 1 thru 8 for remaining samples.
10. Load filtered and marked autosampler vials into autosampler tray.
11. Set up sequence using the PeakNet 6.0 software to mirror placement in tray.

Appendix C: Sonde Sampling Procedure

1. Purge wells using peristaltic pump. NOTE: Stratum A wells can be purged completely, while stratum B and C wells must be purged at least 2 casing volumes from the top of the head.
2. Once recharge is sufficient to submerge the Sonde screened detector probe, lower the detector probe to the bottom of the well, then raise it 1 inch above the bottom. NOTE: It usually takes up to 20 minutes for shallow wells to recharge sufficiently.
3. Allow ample time for detection parameters to equilibrate. This can take up to an hour.
4. Once a steady reading has been reached for all detected parameters, begin collecting data every 5 seconds for 1 minute.
5. Save Data to file in the hand-held device, then move on to the next well.
6. Repeat steps 1 thru 5 for the remainder of the wells.

Appendix D: Dionex Analysis Program for PeakNet 6.0

Time (min)	Item	Value
	Pressure LowerLimit =	200
	Pressure.UpperLimit =	2500
	%A. Equate =	"Water"
	%B. Equate =	"%B"
	%C. Equate =	"%C"
	%D. Equate =	"%D"
	Flush	Volume = 100
	Wait	FlushState
	NeedleHeight =	5
	CutSegmentVolume =	10
	SyringeSpeed =	3
	ColumnTemperature =	30
	Cycle =	0
	Data_Collection_Rate =	2.0
	Temperature_Compensation =	1.7
	Oven_Temperature =	30
	Suppressor_Type =	SRS
	Suppressor_Current =	100
	Flow =	1.50
	%B =	0.0
	%C =	0.0
	%D =	0.0
	Pump.Curve =	5
	WaitForTemperature =	False
	Wait	SamplePrep
- 0.100	; this negative step is for command traffic	
	Concentration =	1.00
	EluentGenerator.Curve =	5
0.000	ECD.Autozero	
	Load	
	Wait	CycleTimeState
	Inject	
	Wait	InjectState
	ECD_1.AcqOn	
	Concentration =	1.00
	EluentGenerator.Curve =	5
8.000	Concentration =	1.000
	EluentGenerator.Curve =	5

28.000	Concentration =	30.000
	EluentGenerator.Curve =	5
33.000	Concentration =	45.000
	EluentGenerator.Curve =	5
33.000	ECD_1.AcqOff	
	Concentration =	45.000
	EluentGenerator.Curve =	5
33.100	Concentration =	1.000
	EluentGenerator.Curve =	5
36.500	Concentration =	1.000
	EluentGenerator.Curve =	5

Wait
End

Appendix E: Acetate, January 2002

ppm Acetate, Strata A

ppm Acetate, Strata B

ppm Acetate, Strata C
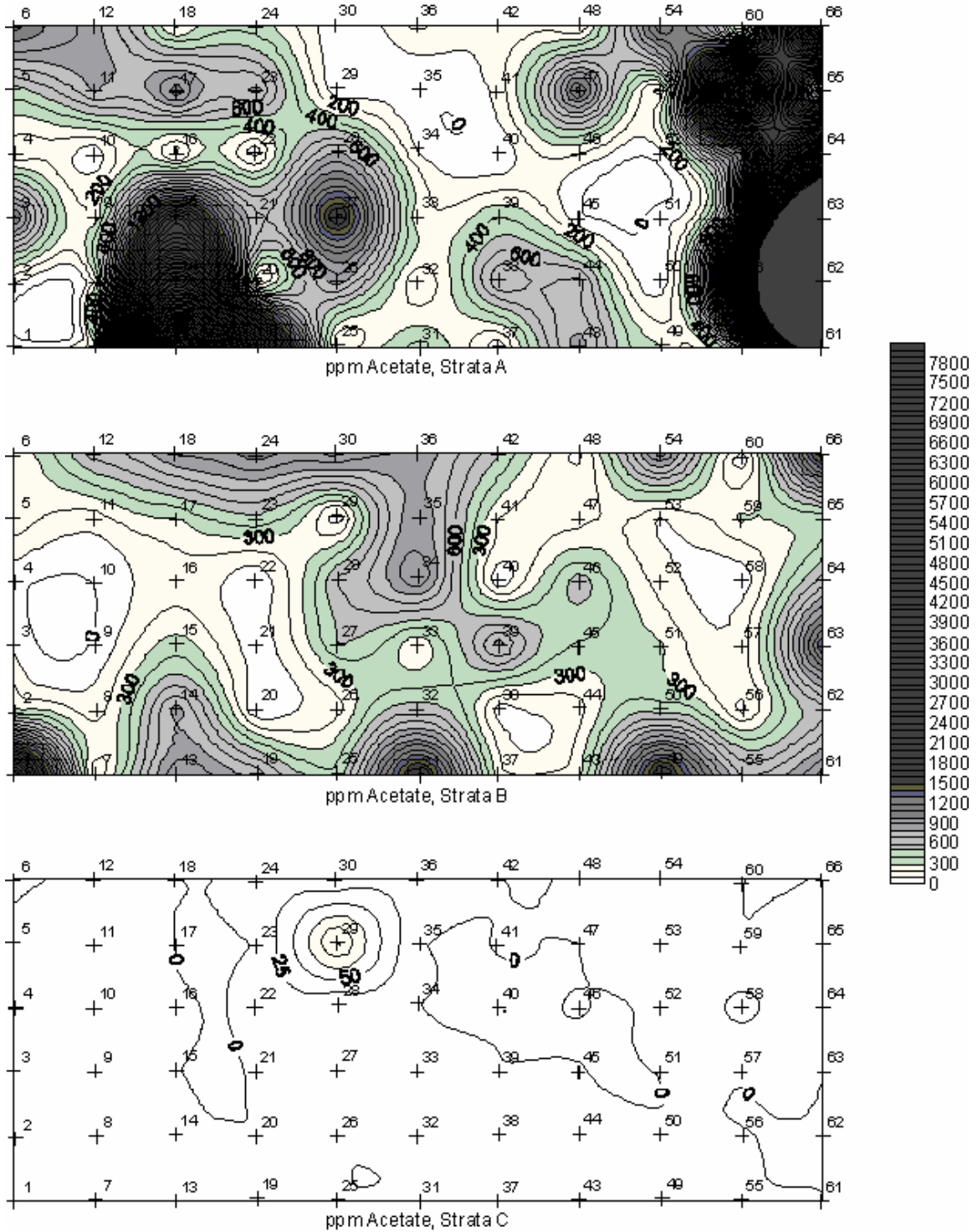
Appendix F: Butyrate, January 2002

ppb Butyrate, Strata A

ppb Butyrate, Strata B

ppb Butyrate, Strata C
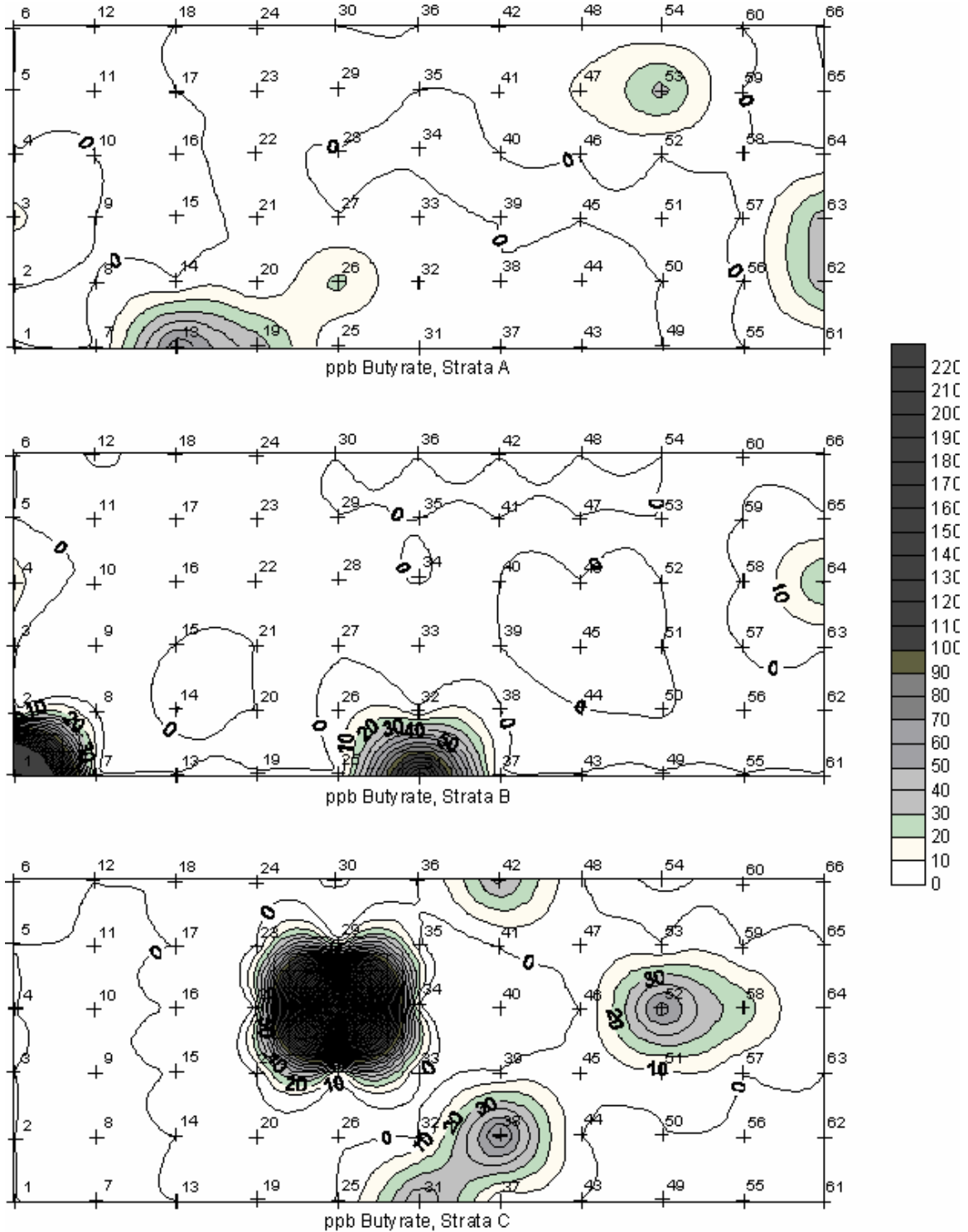
Appendix G: Formate, January 2002

ppm Formate, Strata A

ppm Formate, Strata B

ppm Formate, Strata C
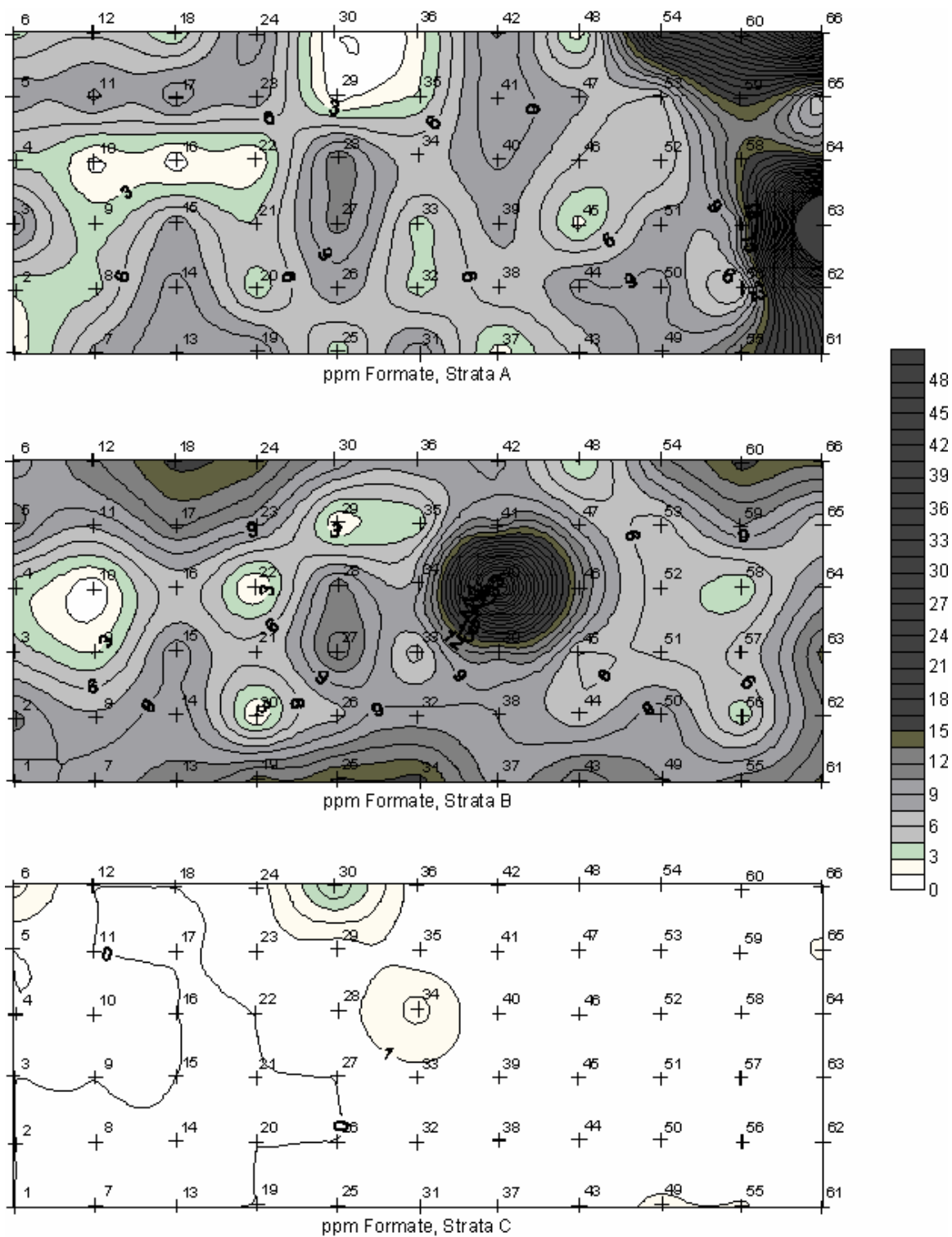
Appendix H: Lactate, January 2002

ppb Lactate, Strata A

ppb Lactate, Strata B

ppb Lactate, Strata C
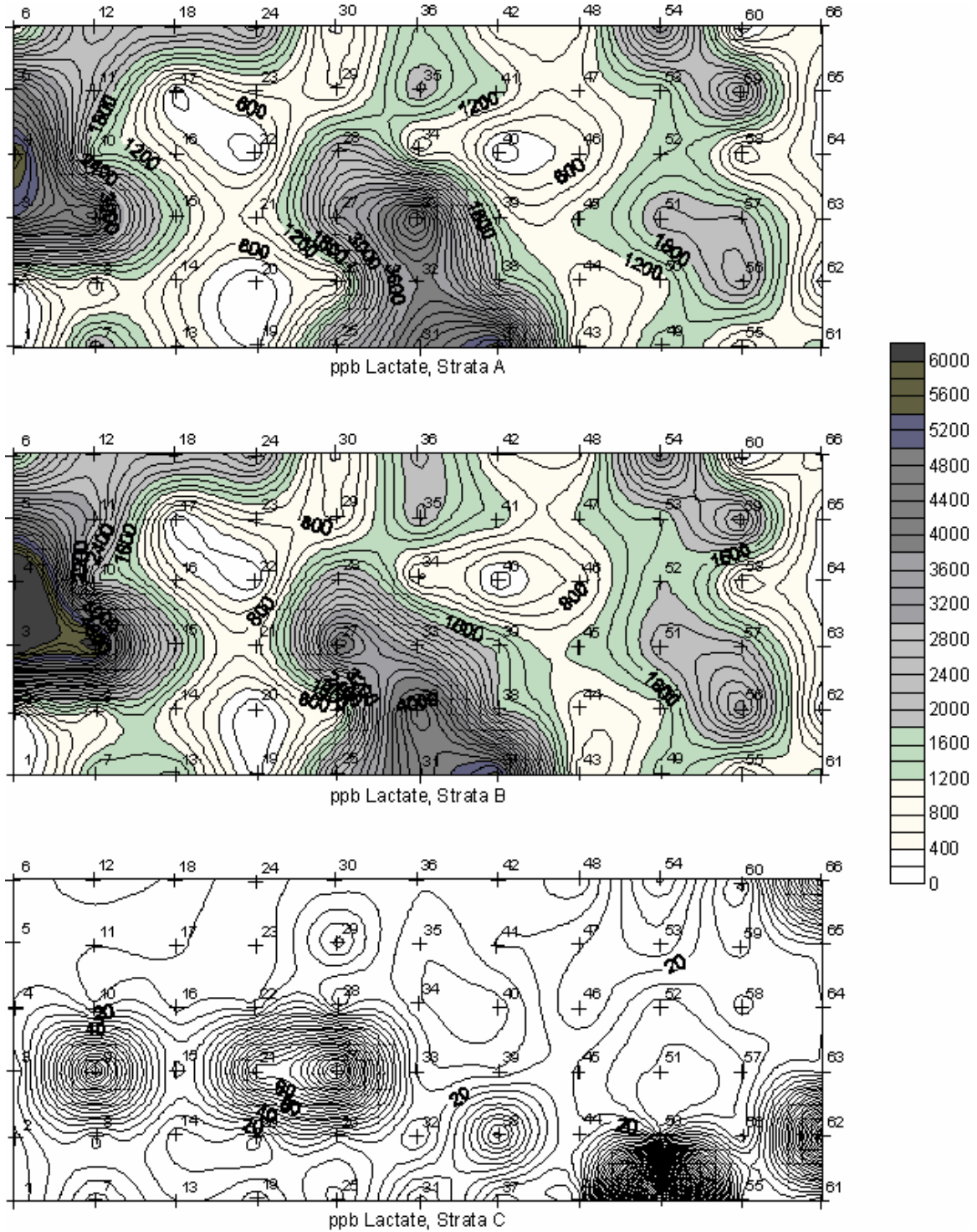
Appendix I: Propionate, January 2002

ppm Propionate, Strata A

ppm Propionate, Strata B

ppm Propionate, Strata C
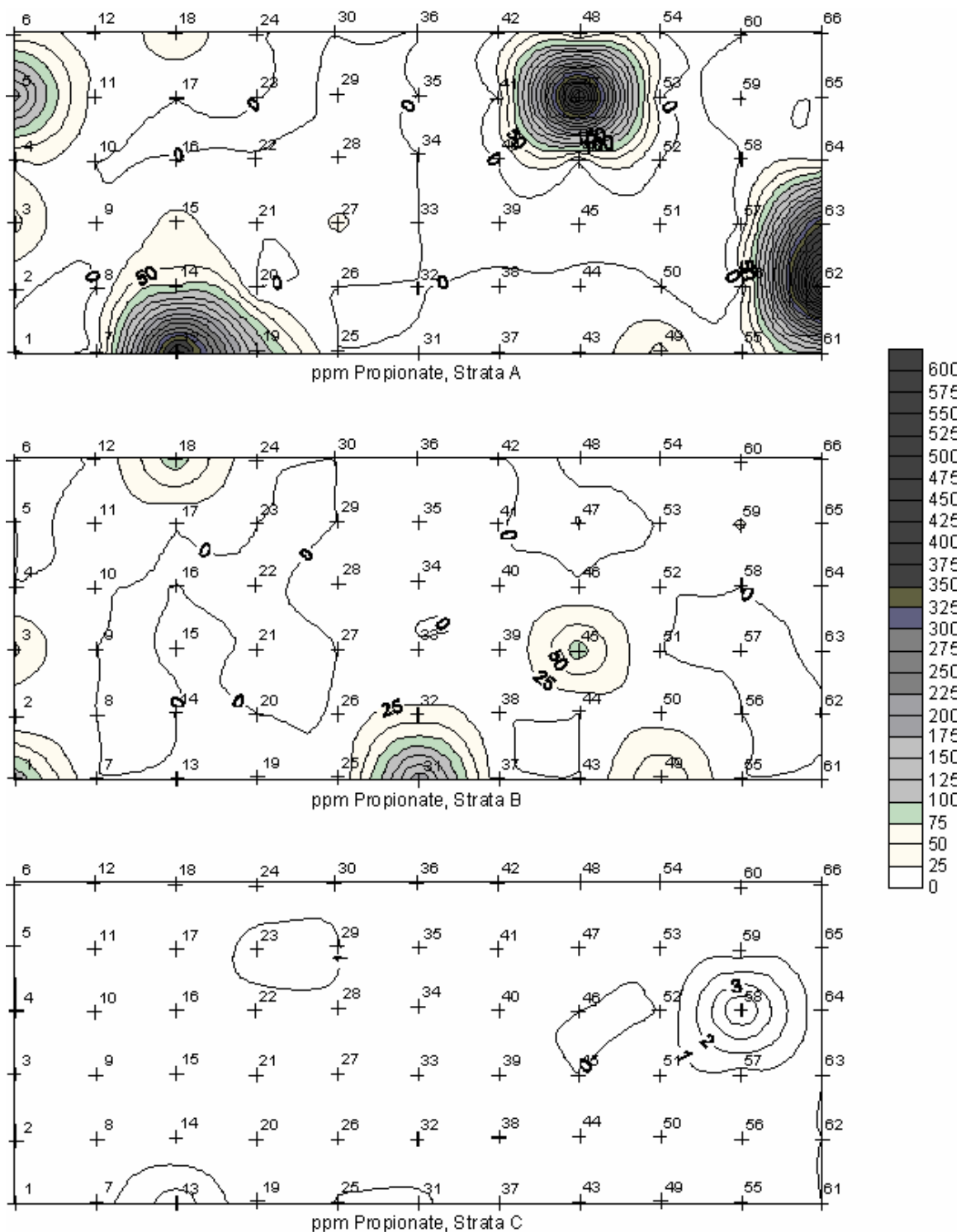
Appendix J: Bromide, January 2002

ppb Bromide, Strata A

ppb Bromide, Strata B

ppb Bromide, Strata C
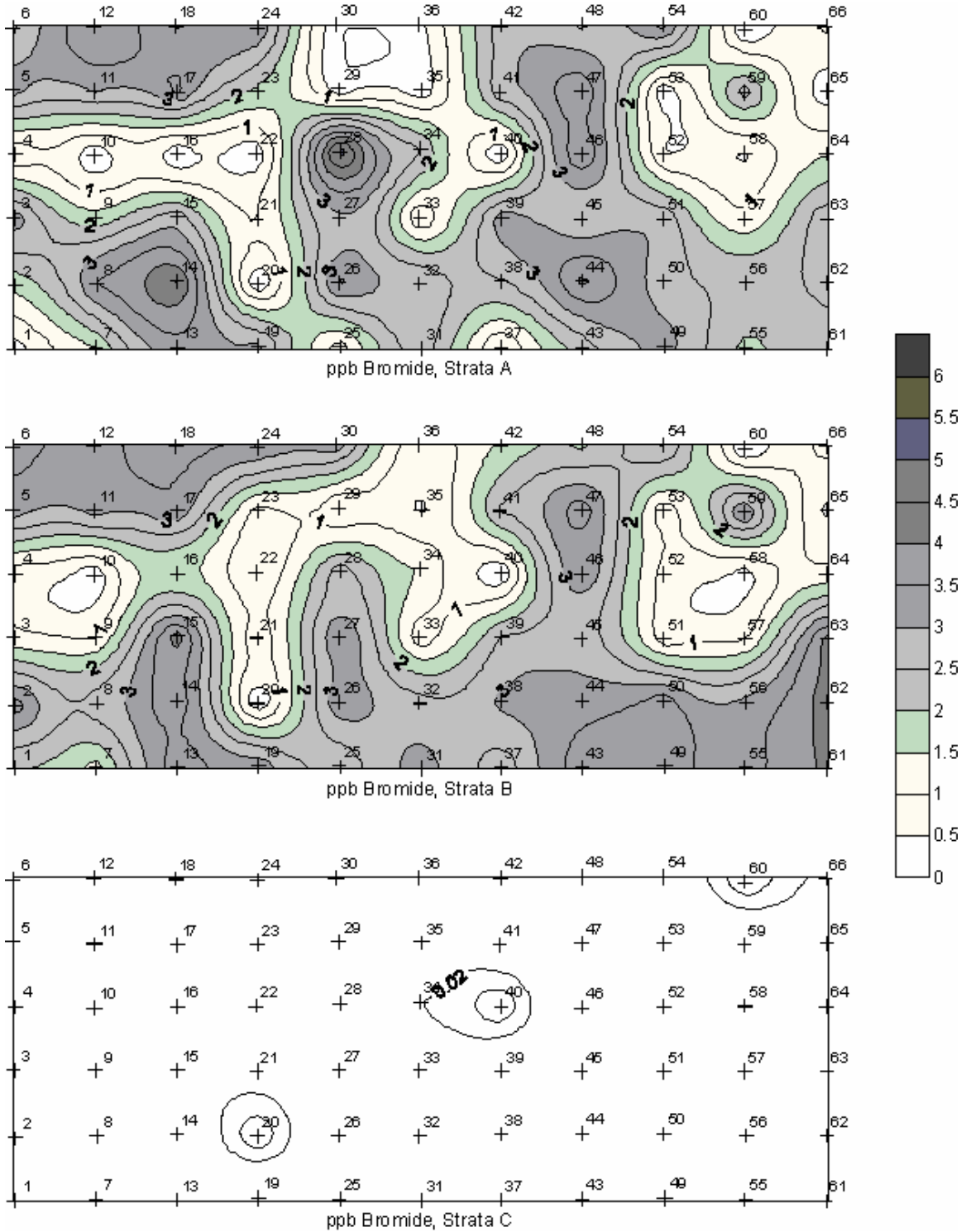
Appendix K: Chloride, January 2002

ppm Chloride, Strata A

ppm Chloride, Strata B

ppm Chloride, Strata C

78
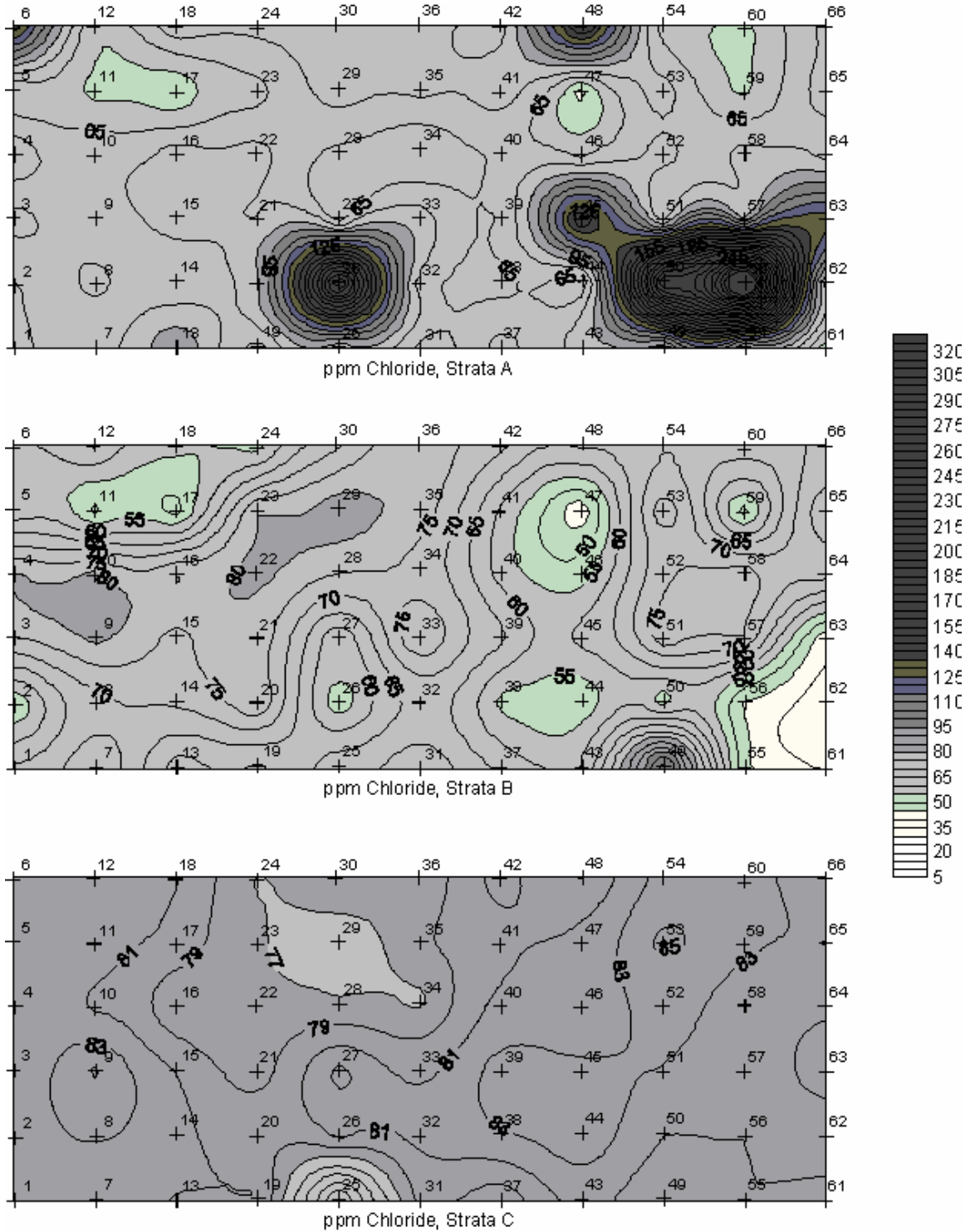
Appendix L: Fluoride, January 2002

ppb Fluoride, Strata A

ppb Fluoride, Strata B

ppb Fluoride, Strata C

79
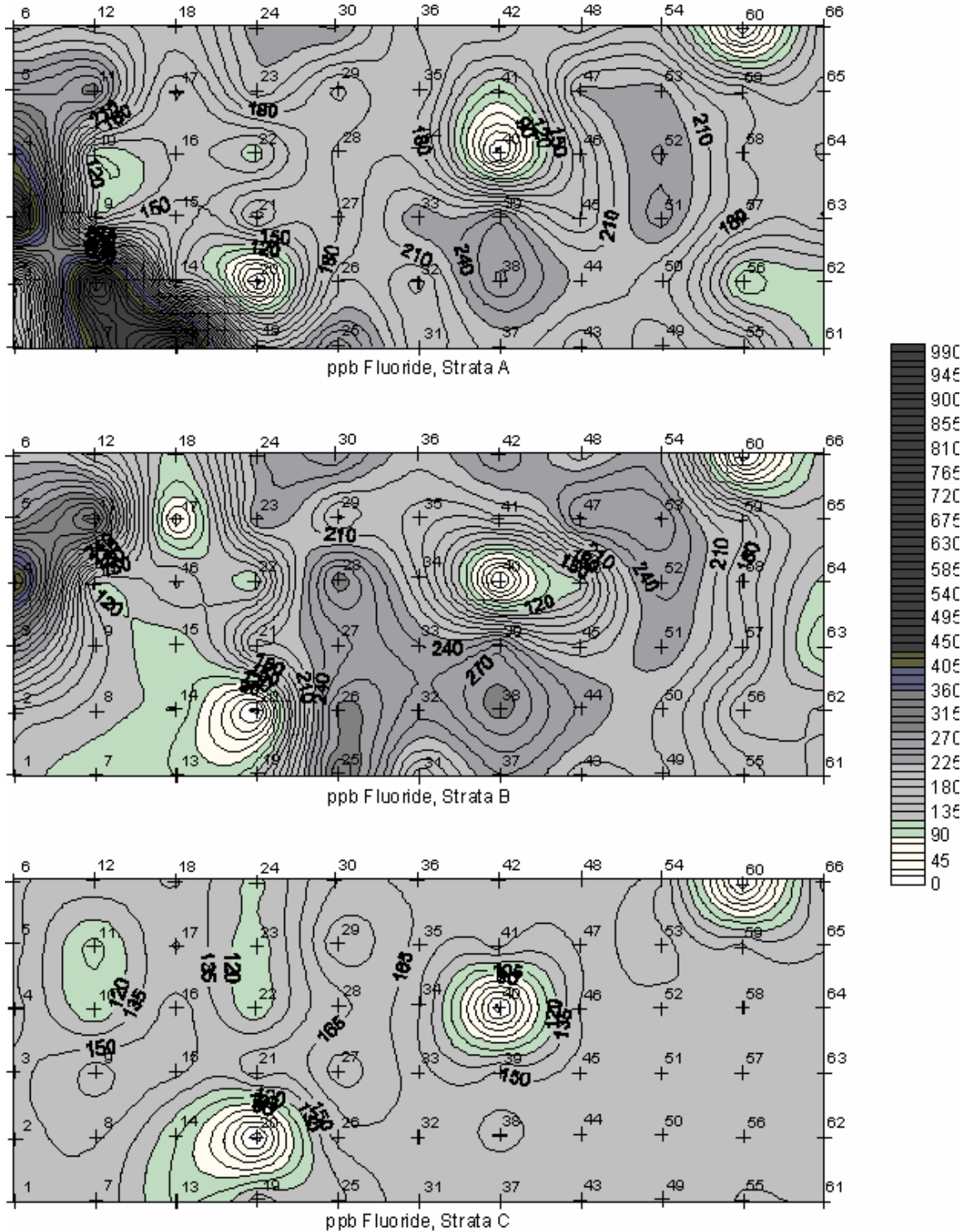
Appendix M: Nitrate, January 2002

ppm Nitrate, Strata A

ppm Nitrate, Strata B

ppm Nitrate, Strata C
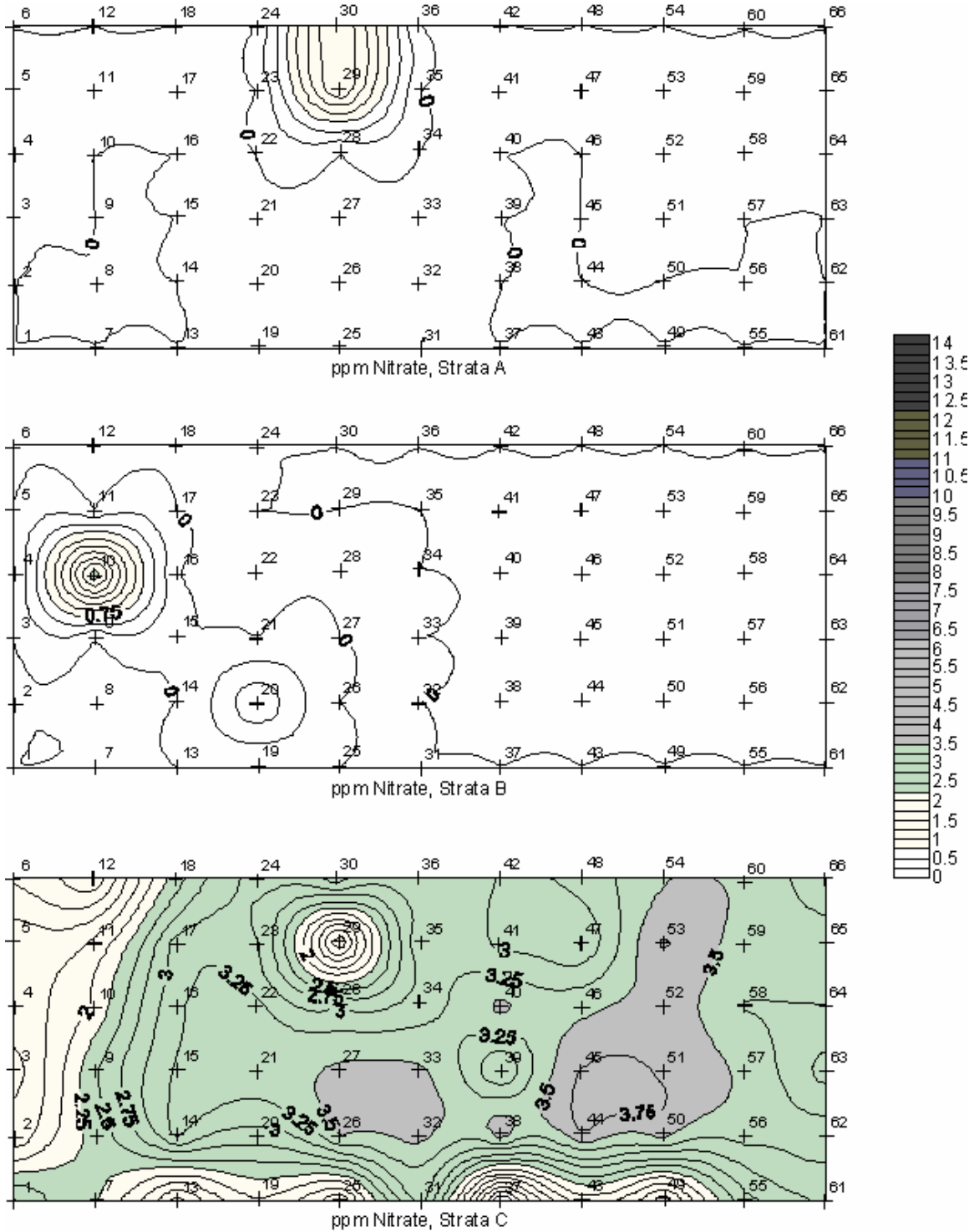
Appendix N: Nitrite, January 2002

ppb Nitrite, Strata A

ppb Nitrite, Strata B

ppb Nitrite, Strata C

81
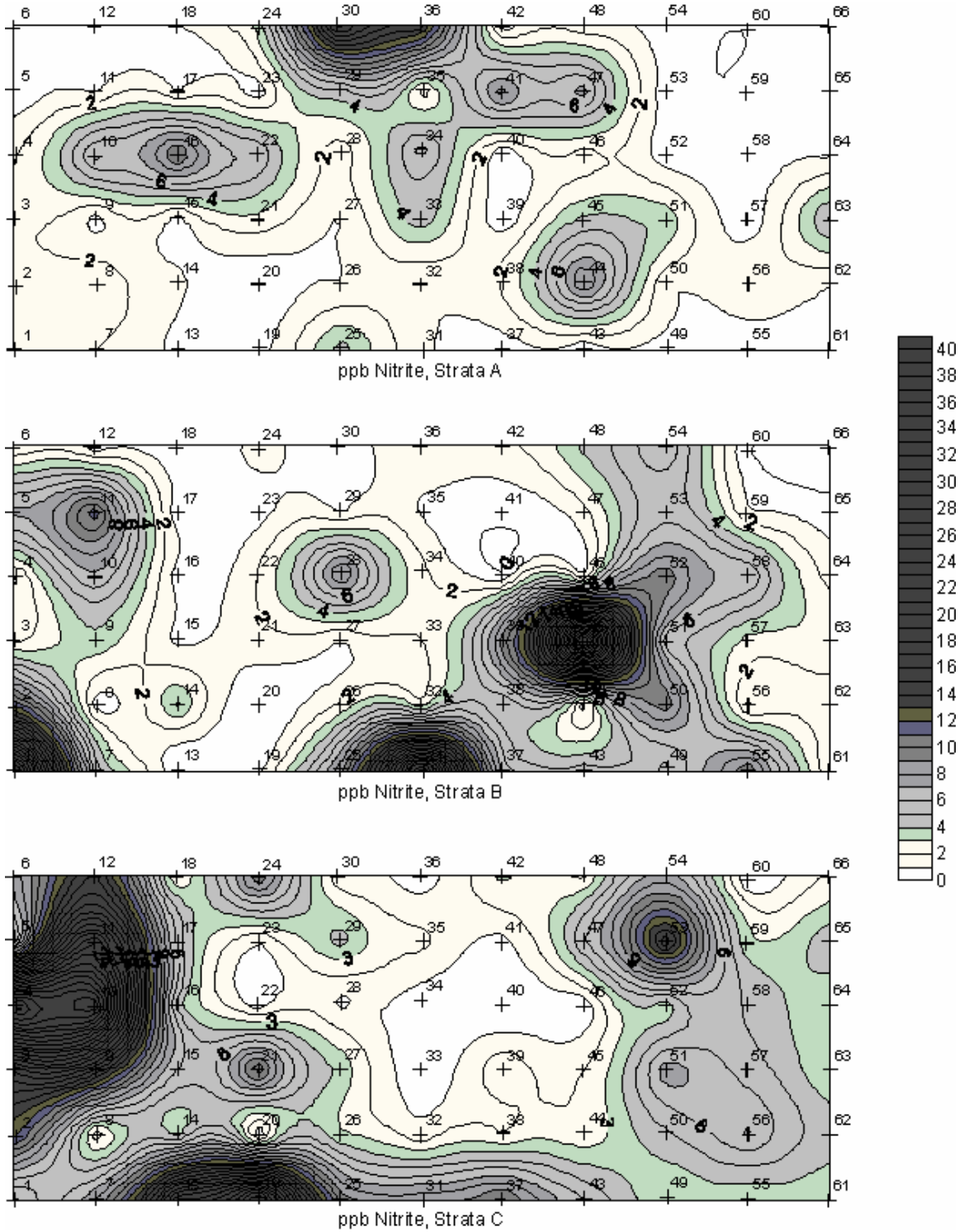
Appendix O: Sulfate, January 2002

ppm Sulfate, Strata A

ppm Sulfate, Strata B

ppm Sulfate, Strata C

82
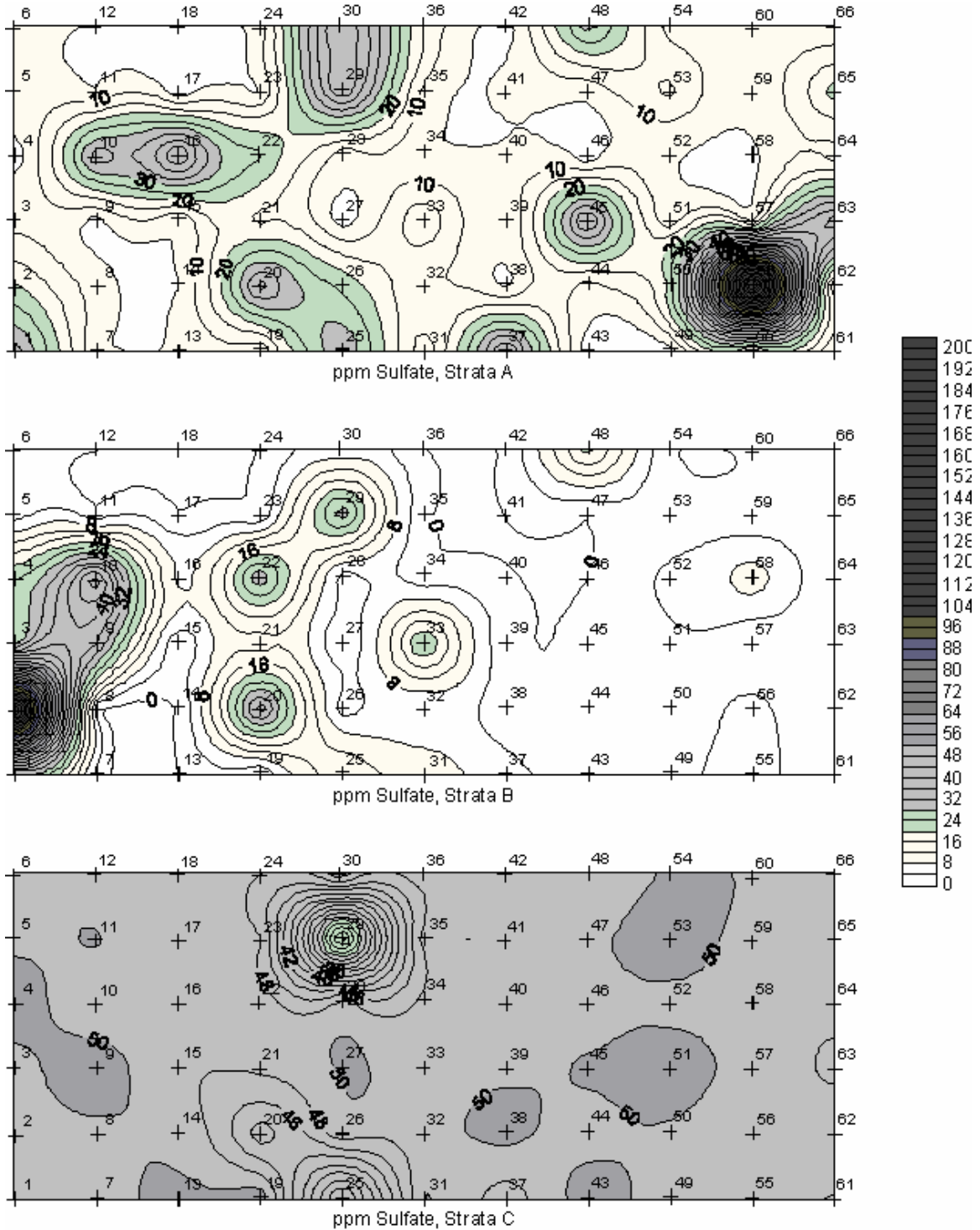
Appendix P: Formate, December 2002 (1)

ppb Formate, Strata A

ppb Formate, Strata B

ppb Formate, Strata C
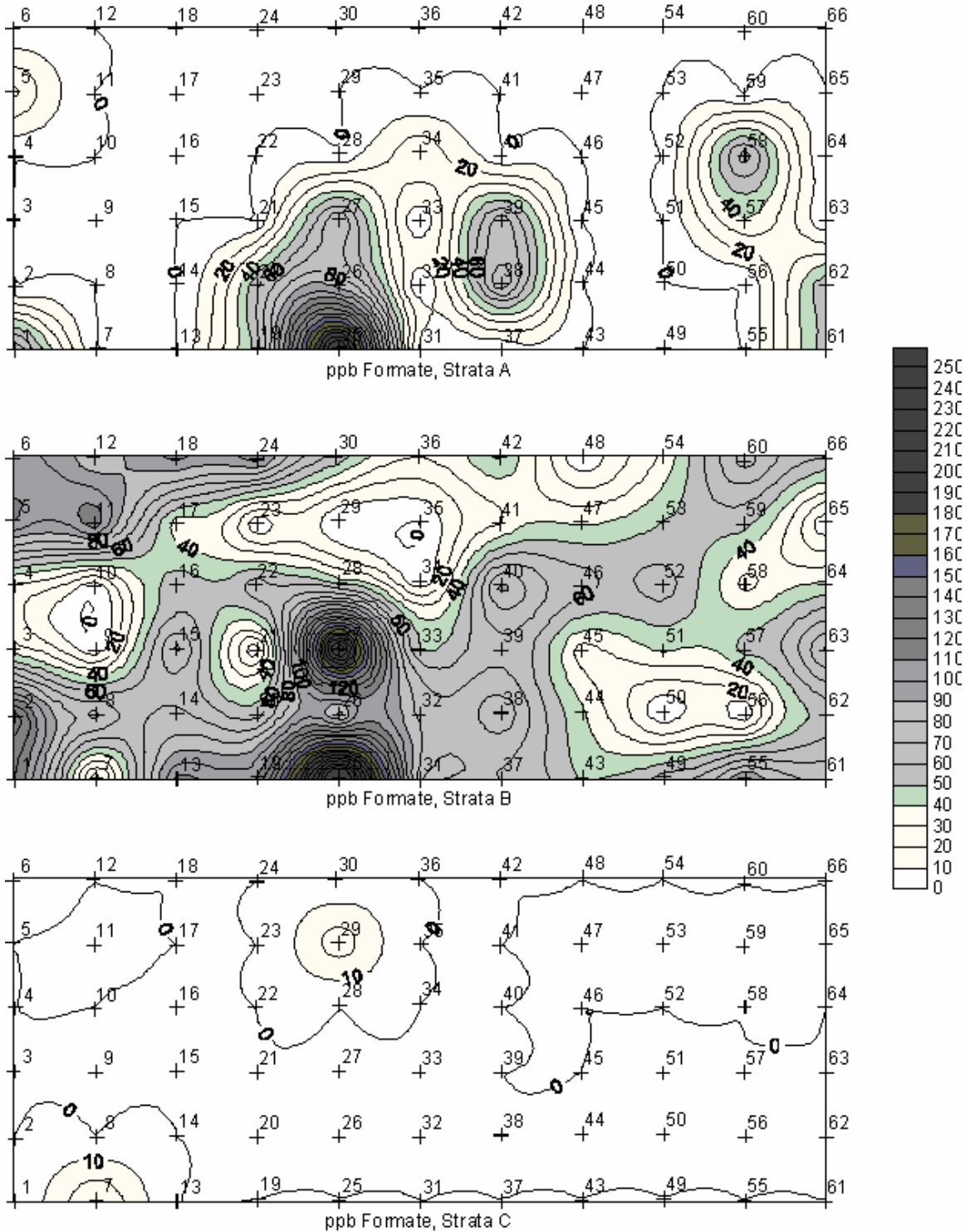
Appendix Q: Formate, December 2002 (2)

ppb Formate, Strata A

ppb Formate, Strata B

ppb Formate, Strata C
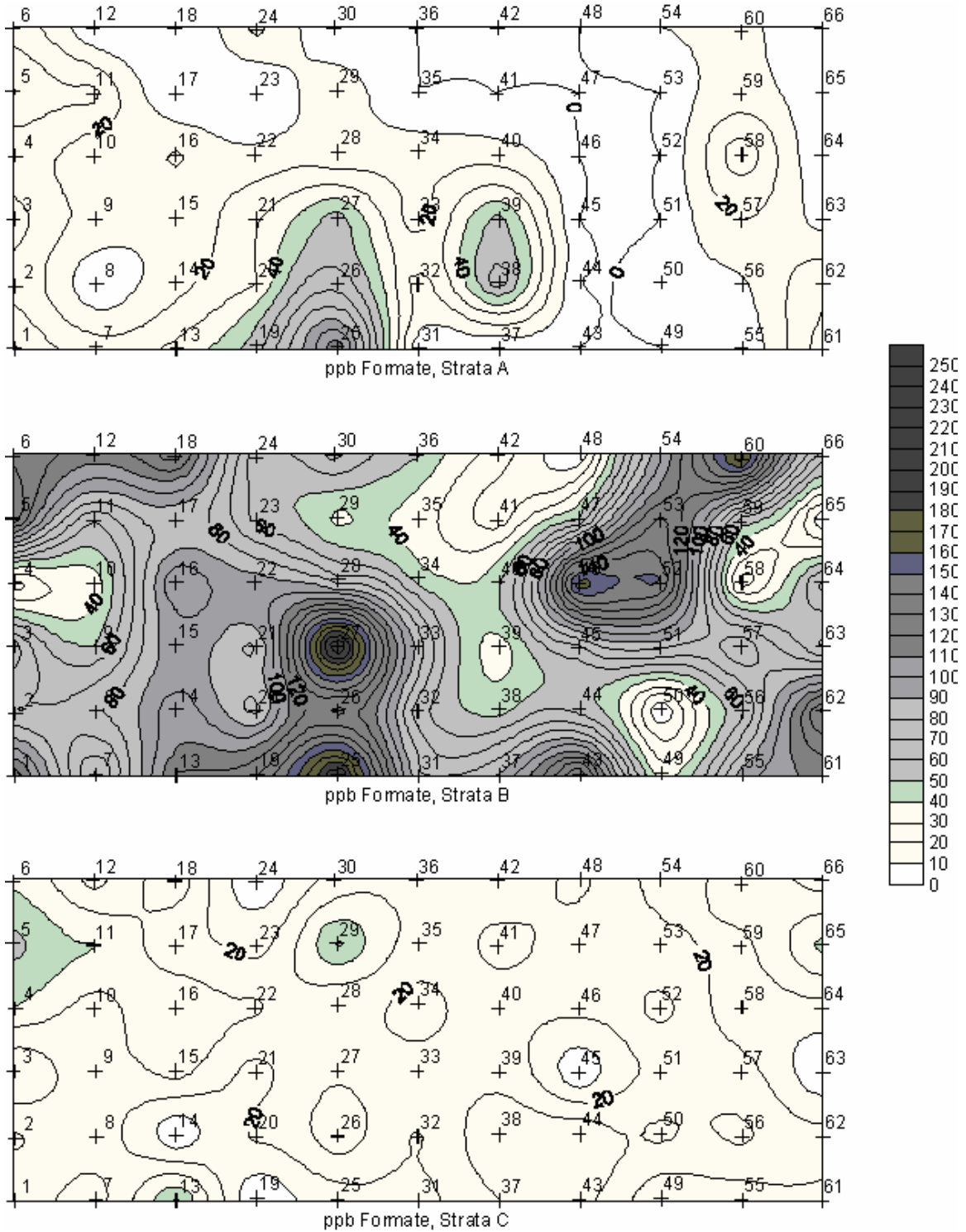
Appendix R: Lactate, December 2002 (1)

ppb Lactate, Strata A

ppb Lactate, Strata B

ppb Lactate, Strata C
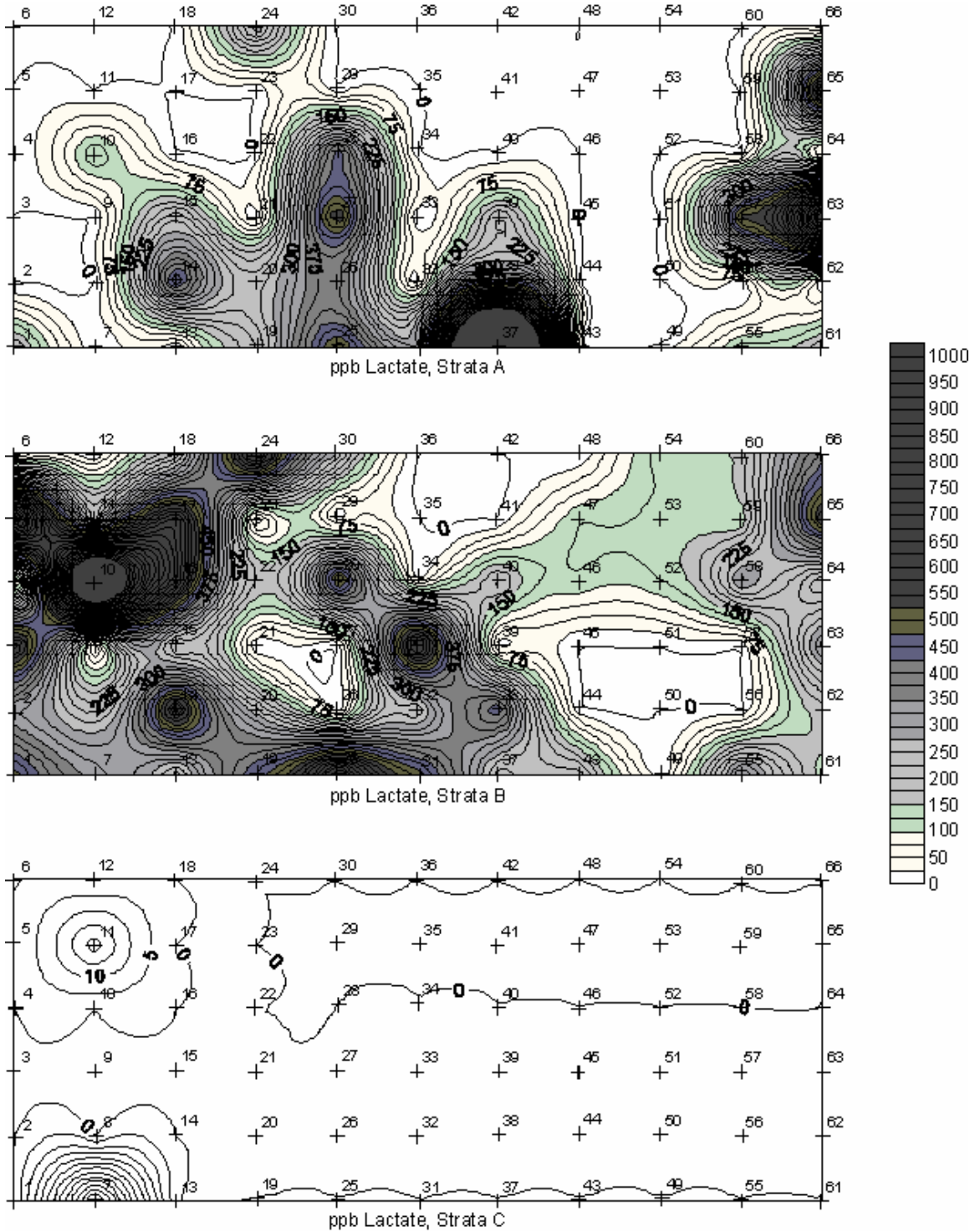
Appendix S: Lactate, December 2002 (2)

ppb Lactate, Strata A

ppb Lactate, Strata B

ppb Lactate, Strata C
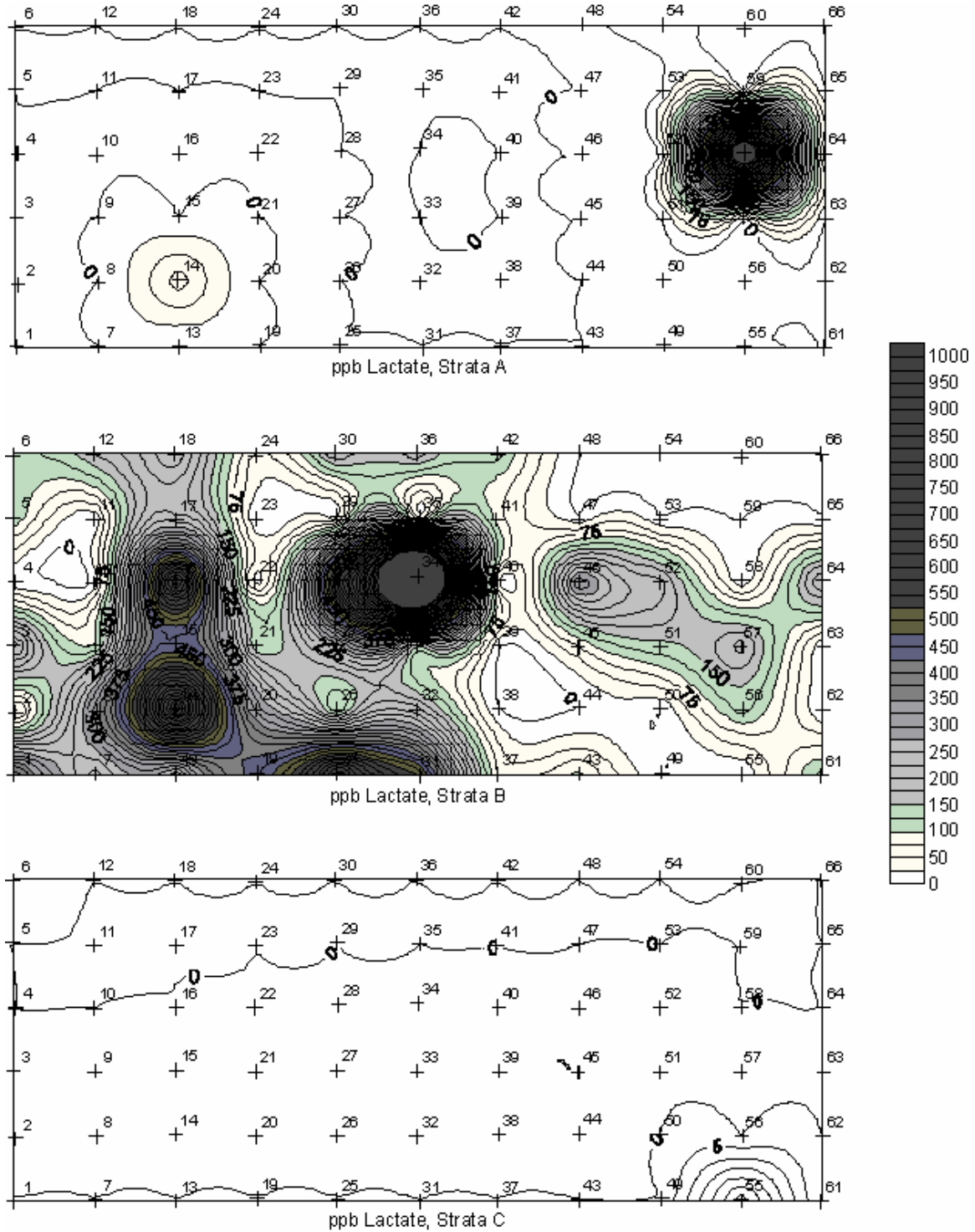
Appendix T: Bromide, December 2002 (1)

ppm Bromide, Strata A

ppm Bromide, Strata B

ppm Bromide, Strata C
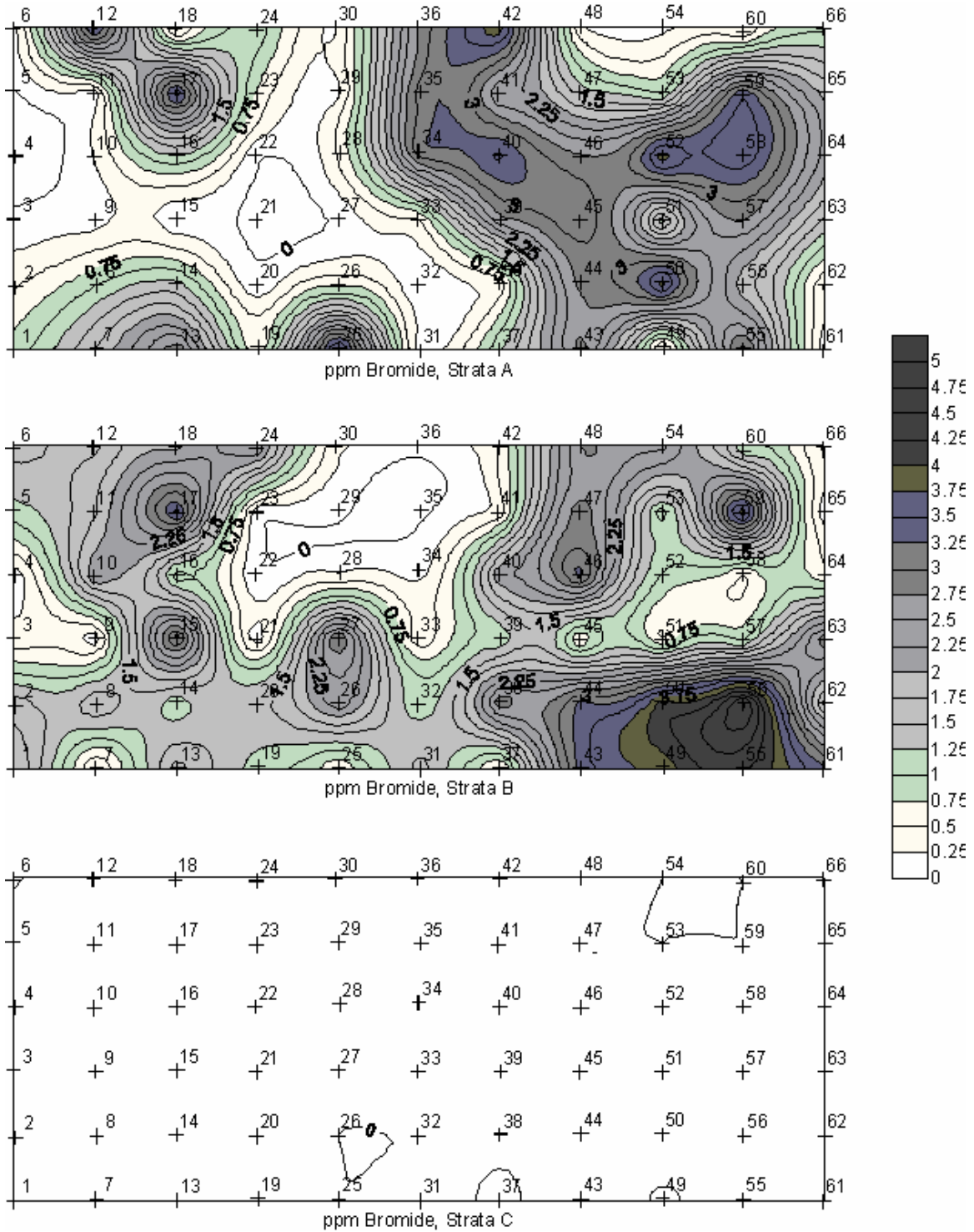
Appendix U: Bromide, December 2002 (2)

ppm Bromide, Strata A

ppm Bromide, Strata B

ppm Bromide, Strata C
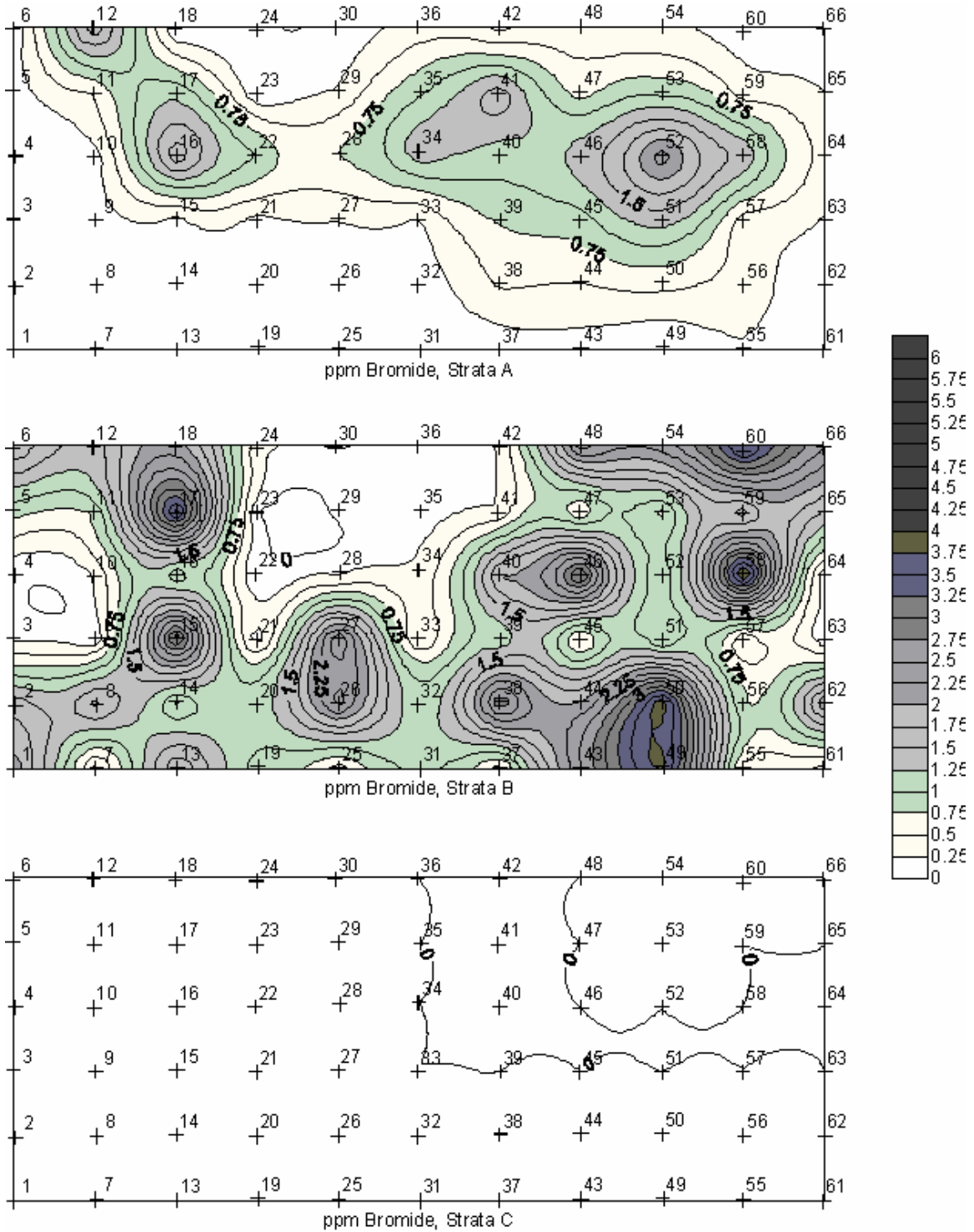
Appendix V: Chloride, December 2002 (1)

ppm Chloride, Strata A

ppm Chloride, Strata B

ppm Chloride, Strata C

89
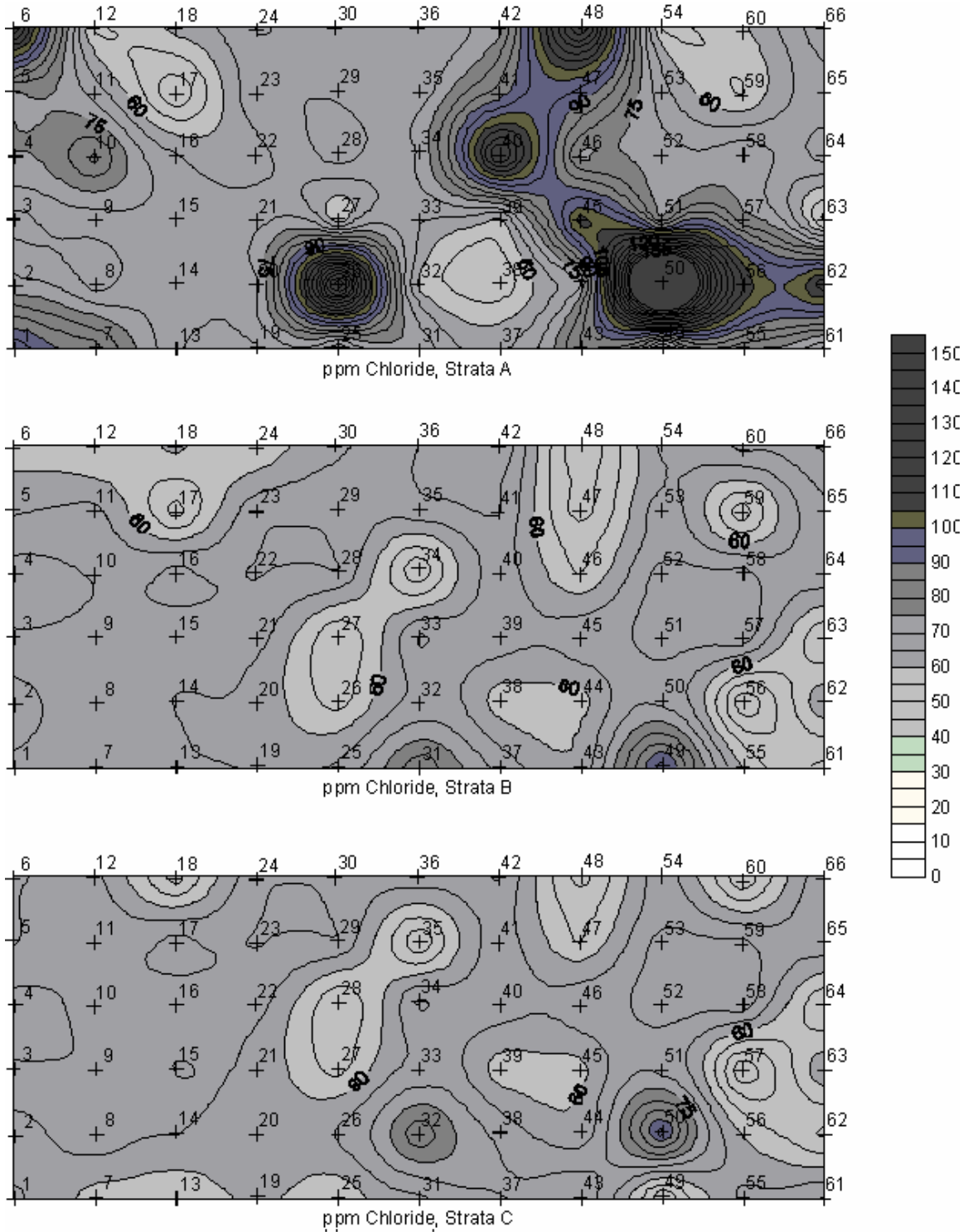
Appendix W: Chloride, December 2002 (2)

ppm Chloride, Strata A

ppm Chloride, Strata B

ppm Chloride, Strata C

ppb Fluoride, Strata A

ppb Fluoride, Strata B

ppb Fluoride, Strata C
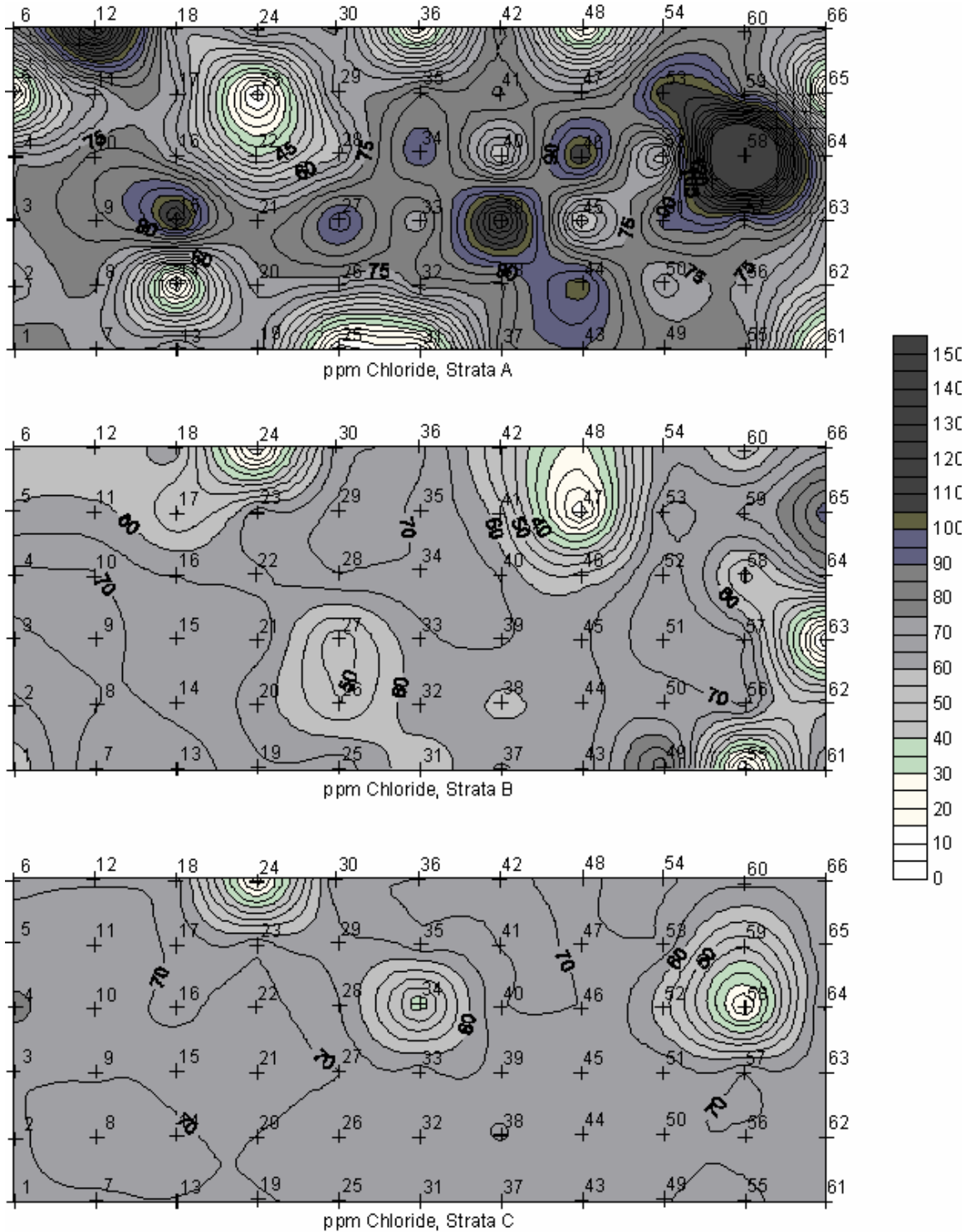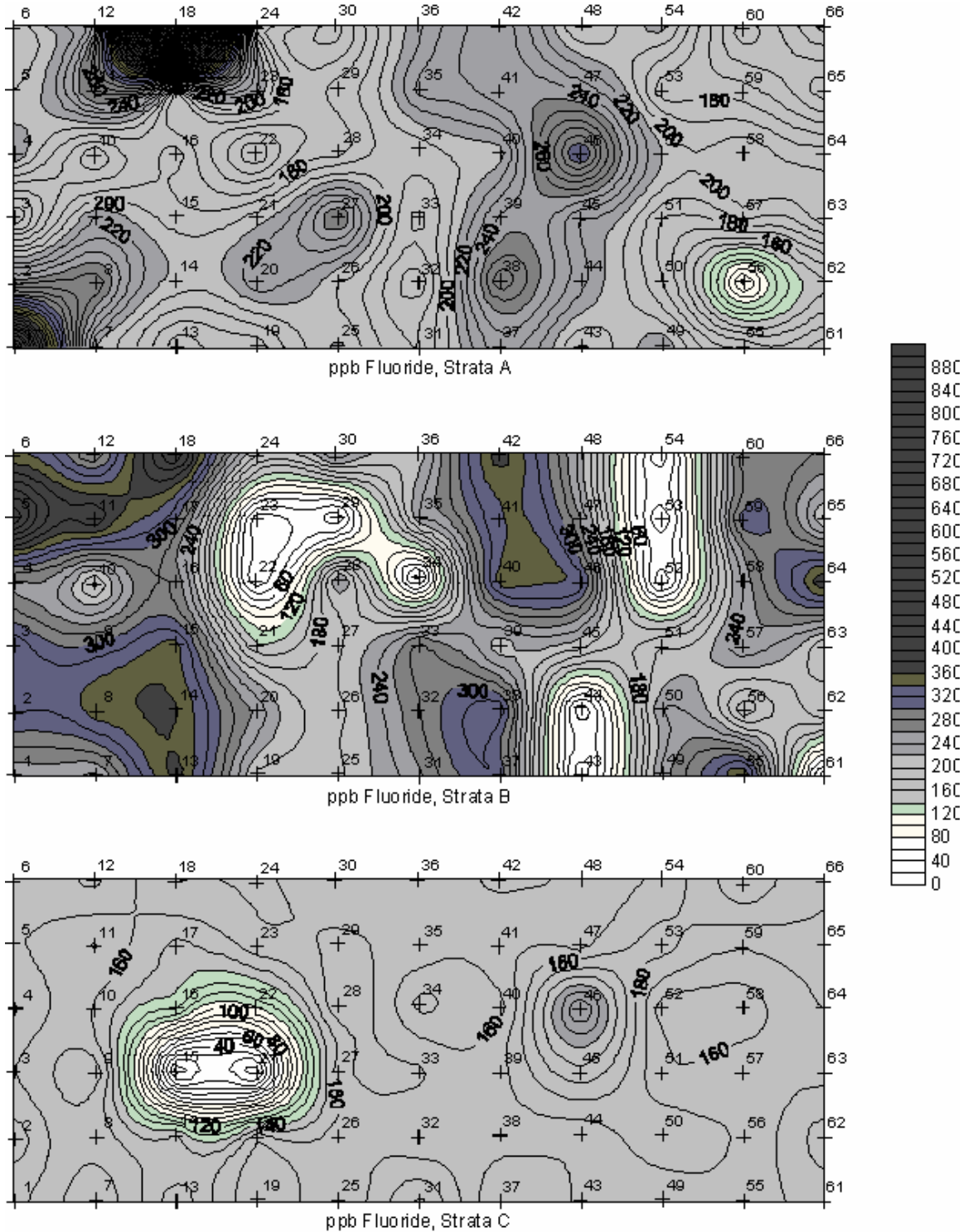
Appendix Y: Fluoride, December 2002 (2)

ppb Fluoride, Strata A

ppb Fluoride, Strata B

ppb Fluoride, Strata C
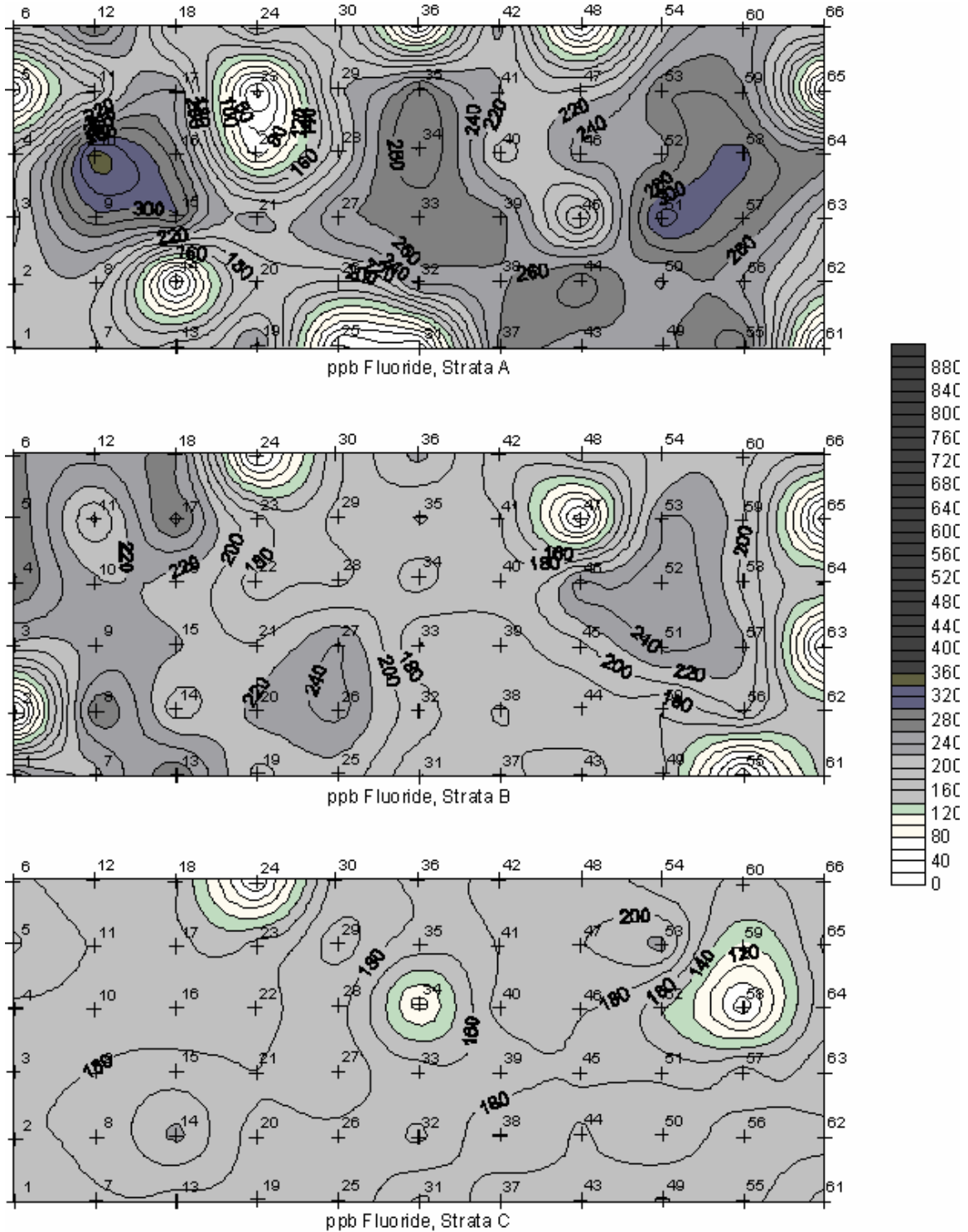
Appendix Z: Nitrate, December 2002 (1)

ppm Nitrate, Strata A

ppm Nitrate, Strata B

ppm Nitrate, Strata C
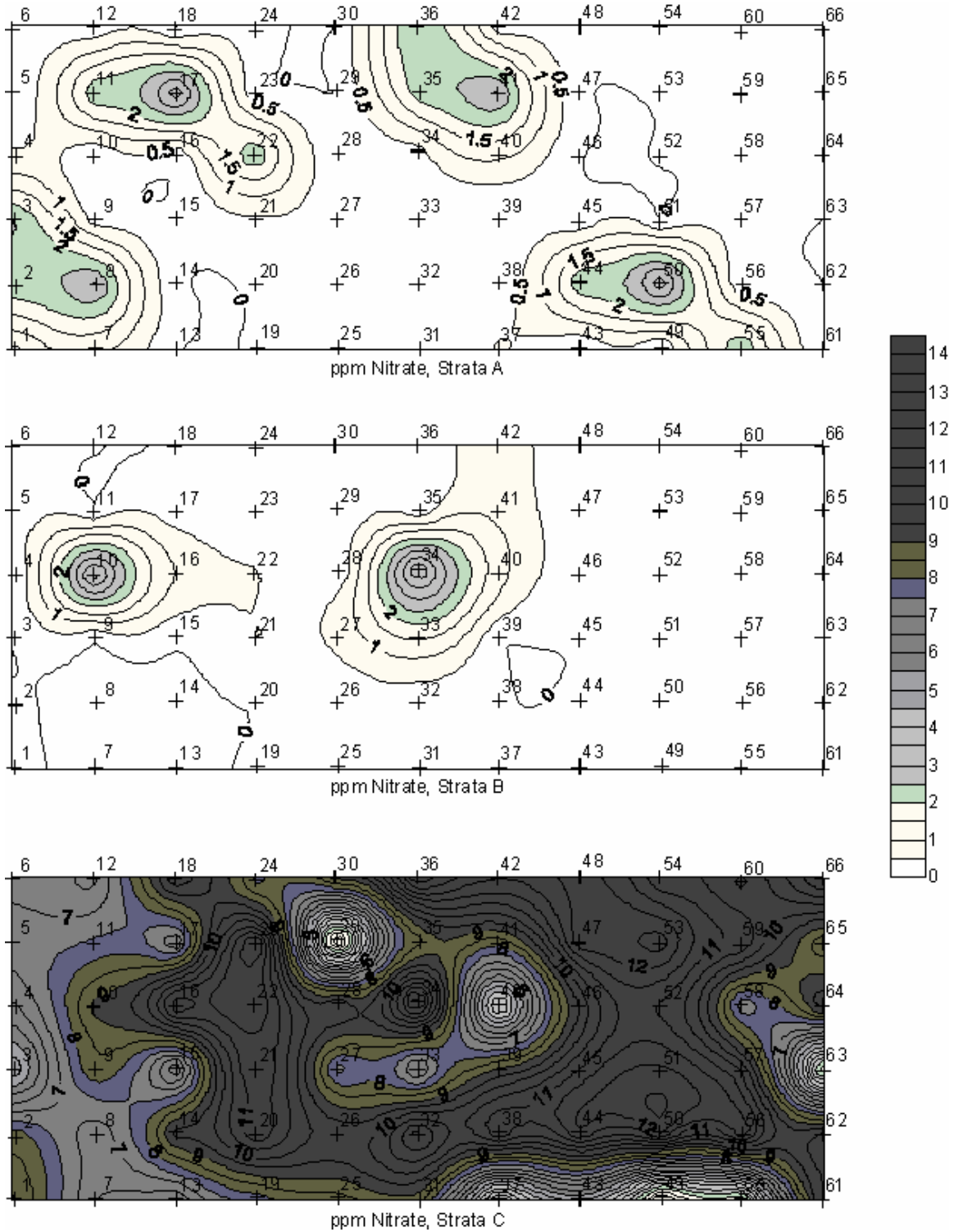
Appendix AA: Nitrate, December 2002 (2)

ppm Nitrate, Strata A

ppm Nitrate, Strata B

ppm Nitrate, Strata C
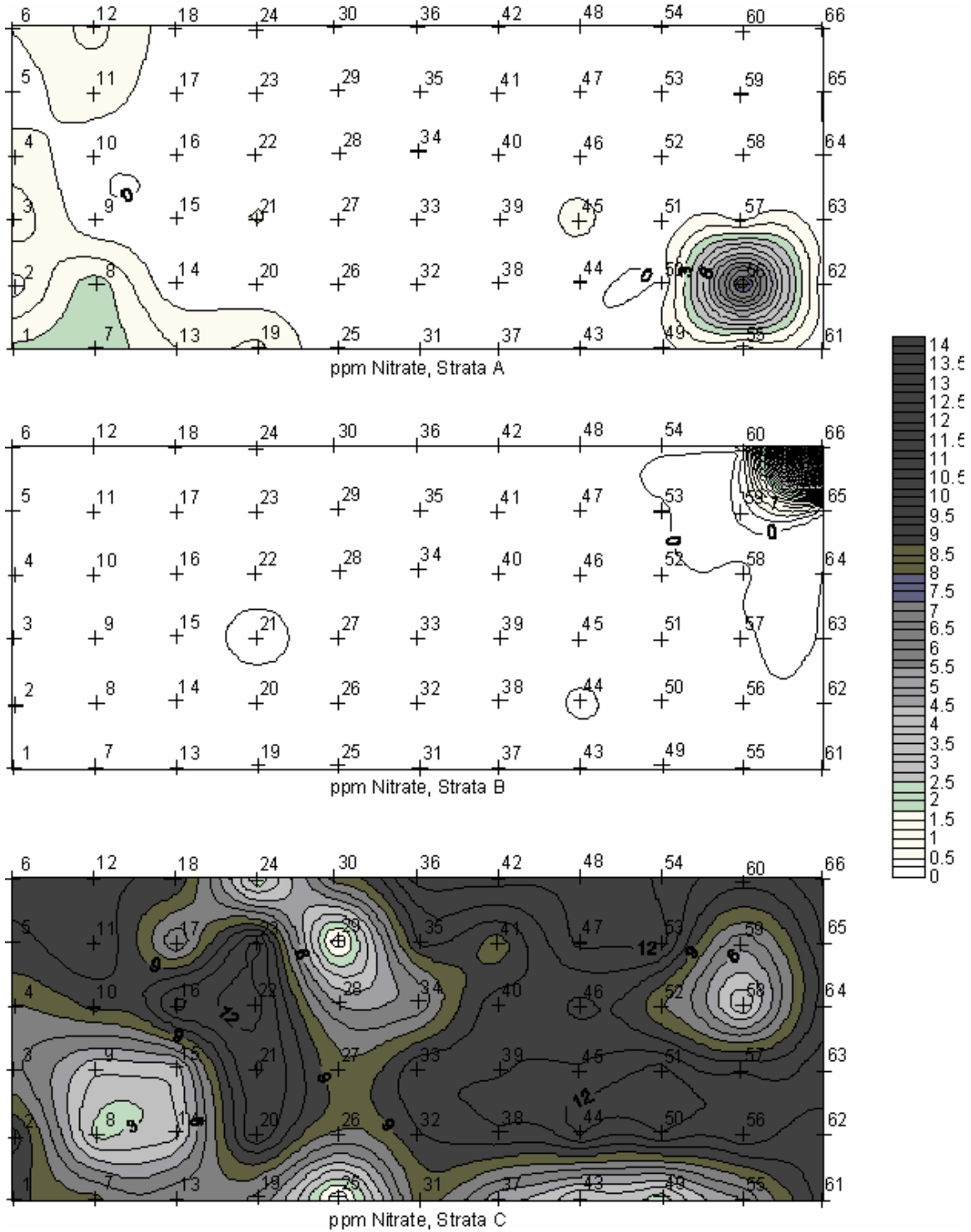
Appendix AB: Nitrite, December 2002 (1)

ppb Nitrite, Strata A

ppb Nitrite, Strata B

ppb Nitrite, Strata C
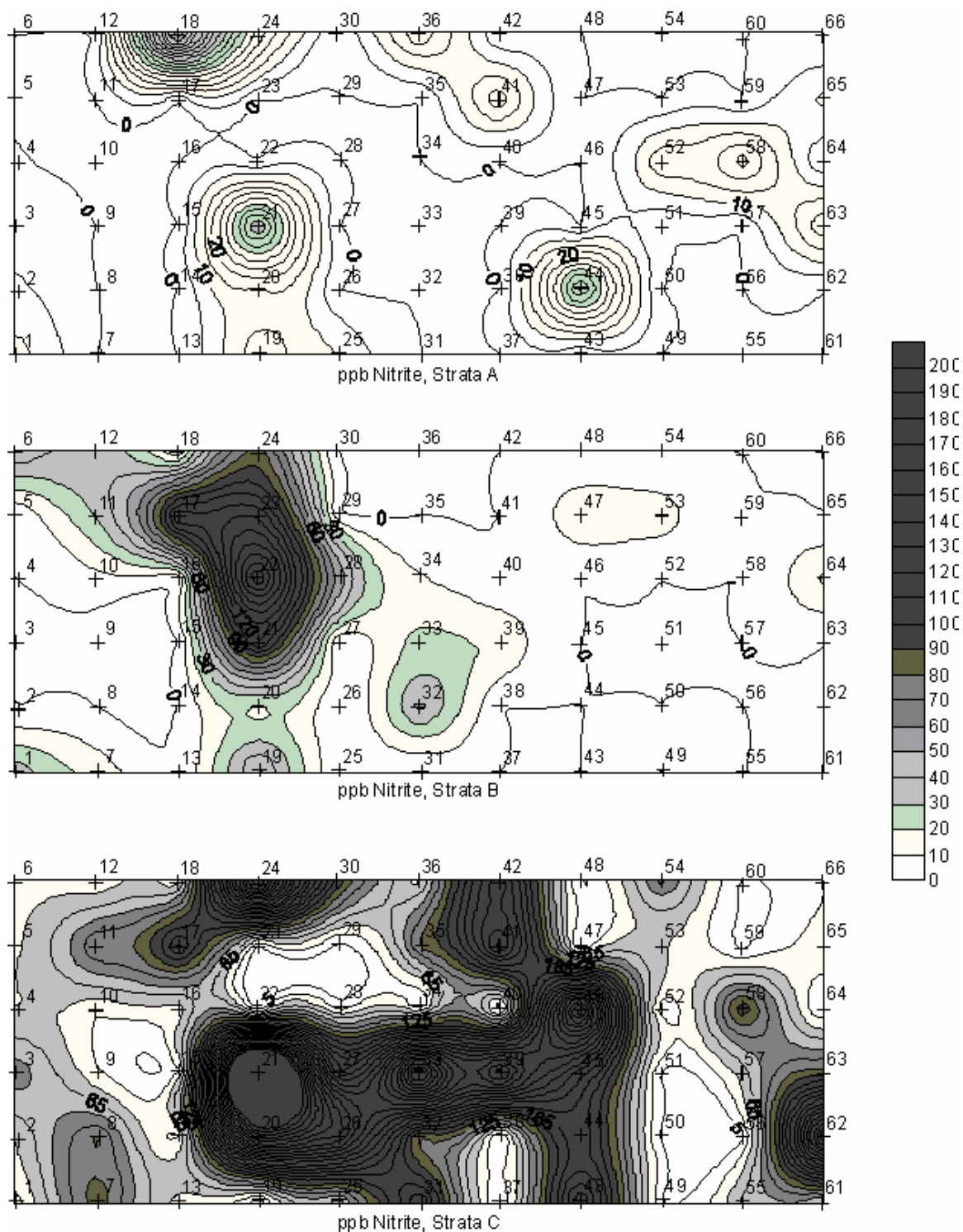
Appendix AC: Nitrite, December 2002 (2)

ppb Nitrite, Strata A

ppb Nitrite, Strata B

ppb Nitrite, Strata C
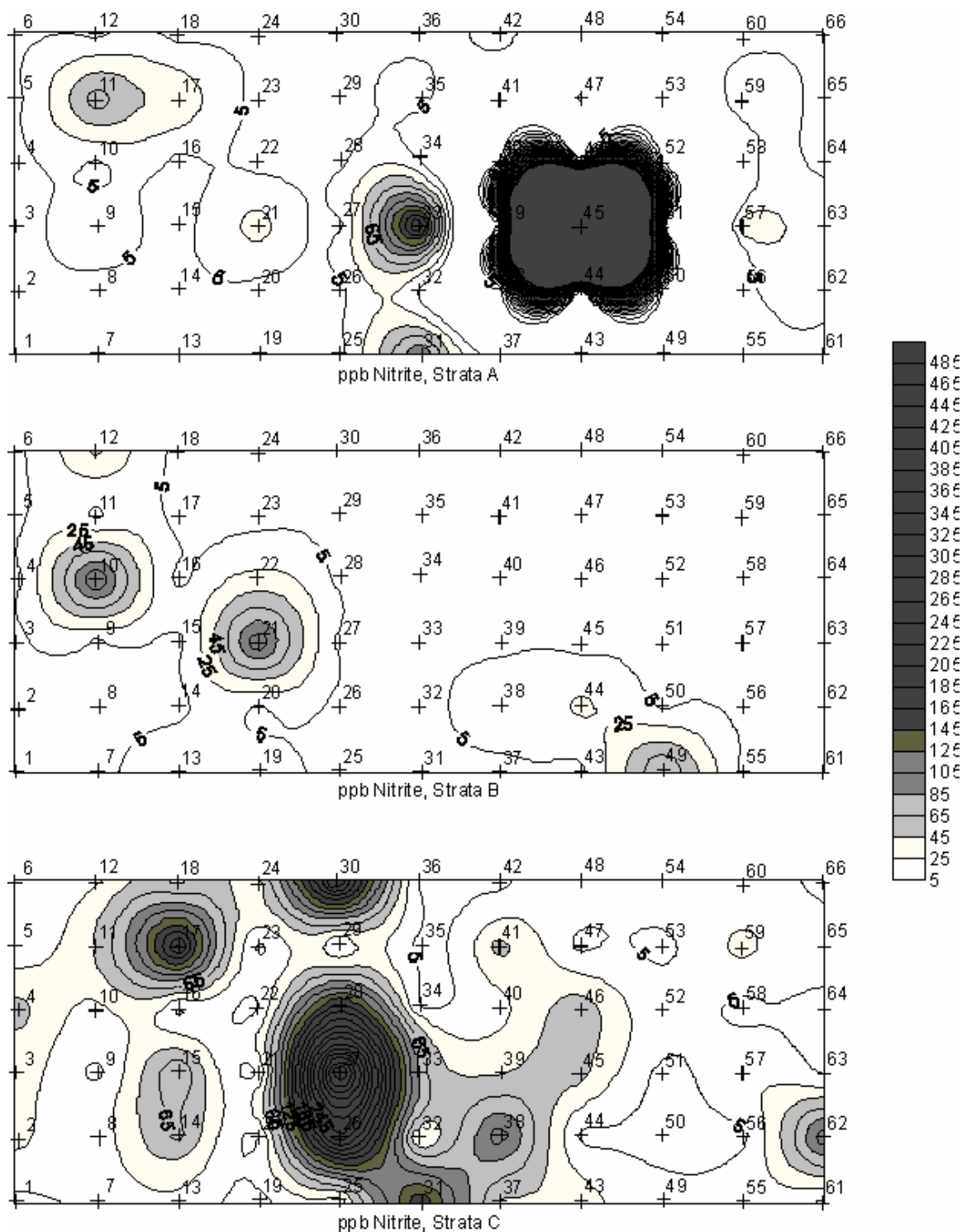
Appendix AD: Sulfate, December 2002 (1)

ppm Sulfate, Strata A

ppm Sulfate, Strata B

ppm Sulfate, Strata C

ppm Sulfate, Strata A

ppm Sulfate, Strata B

ppm Sulfate, Strata C
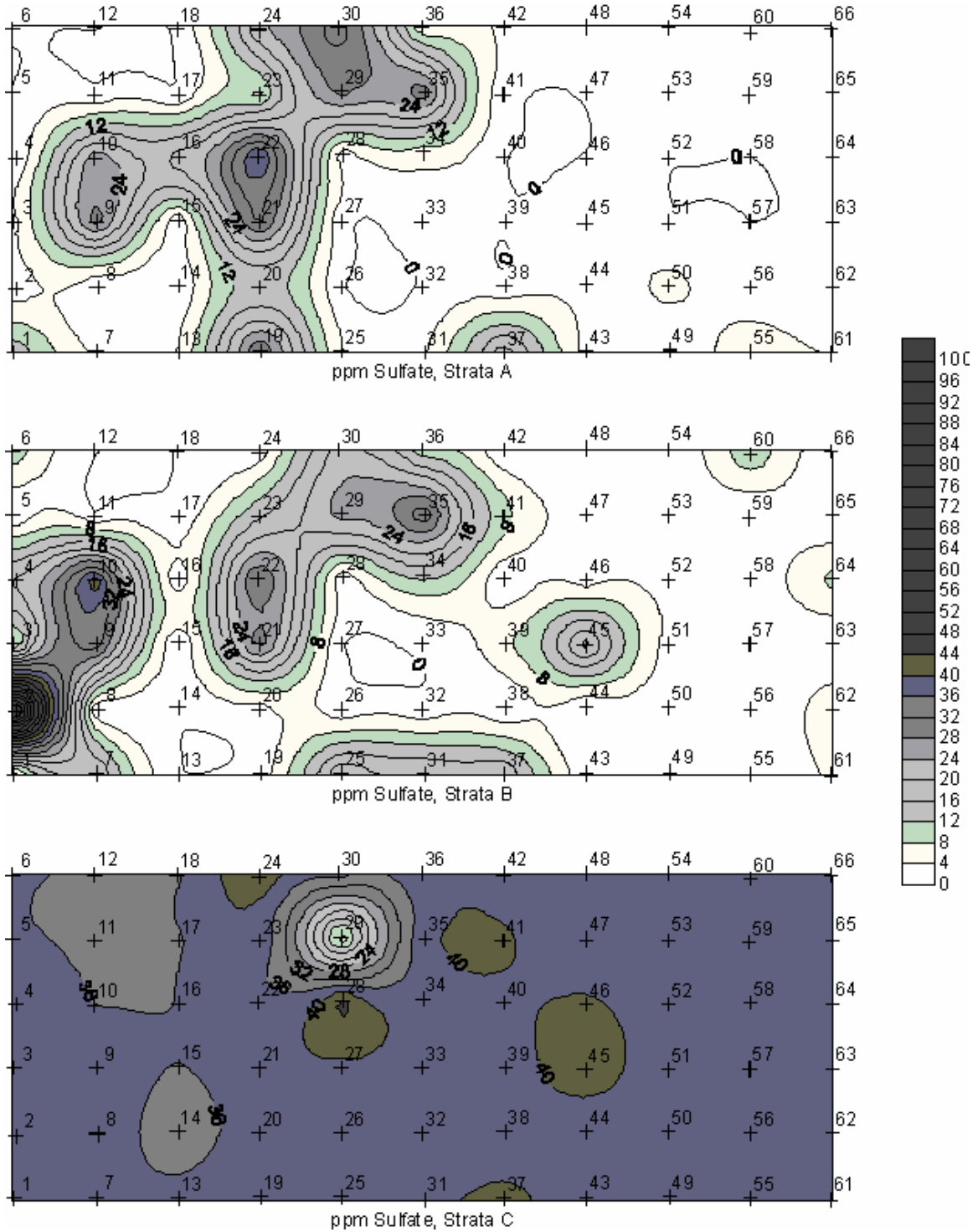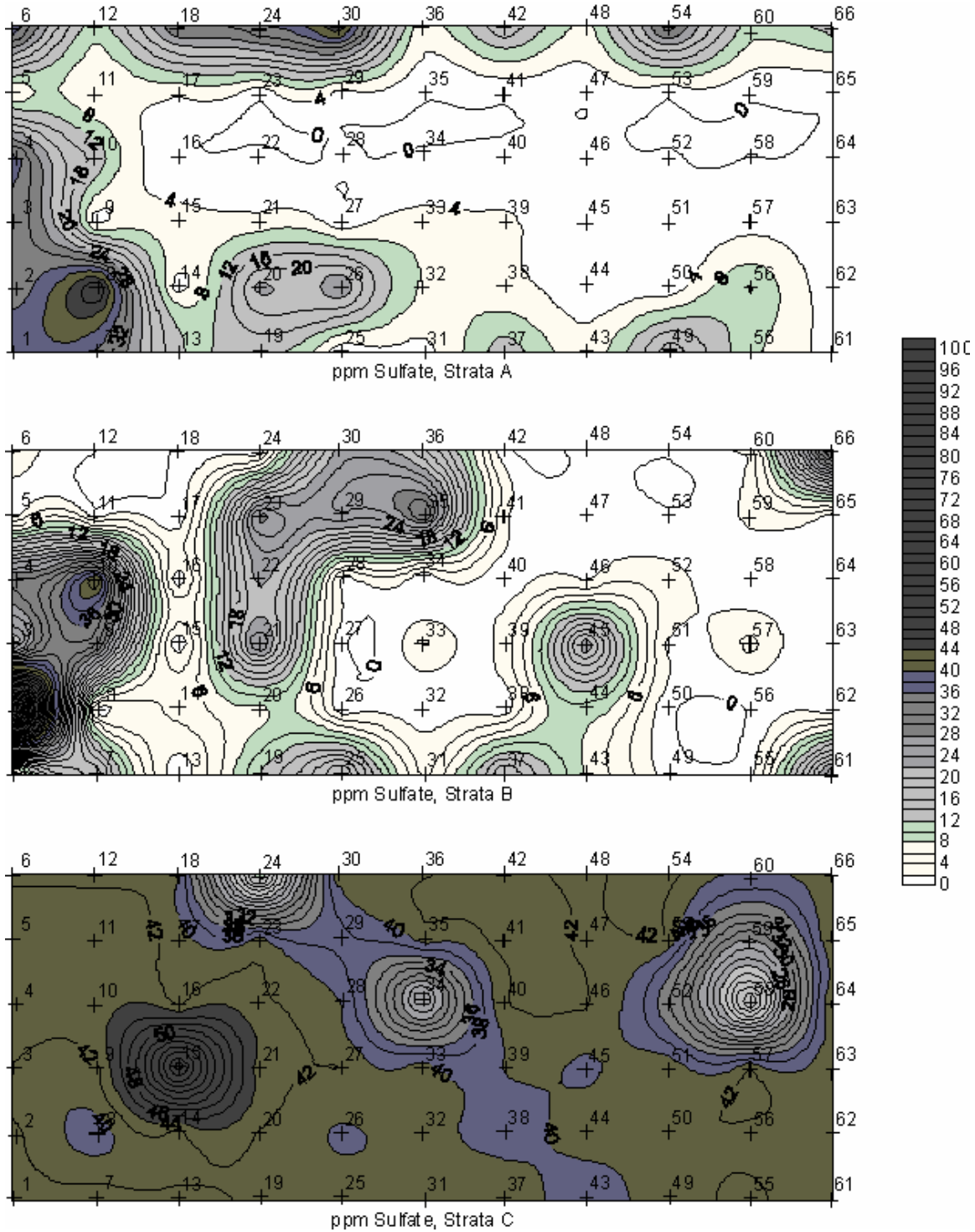
Appendix AF: Dissolved Oxygen, 23 December 2002

mg/L Dissolved Oxygen, Strata A

mg/L Dissolved Oxygen, Strata B

mg/L Dissolved Oxygen, Strata C
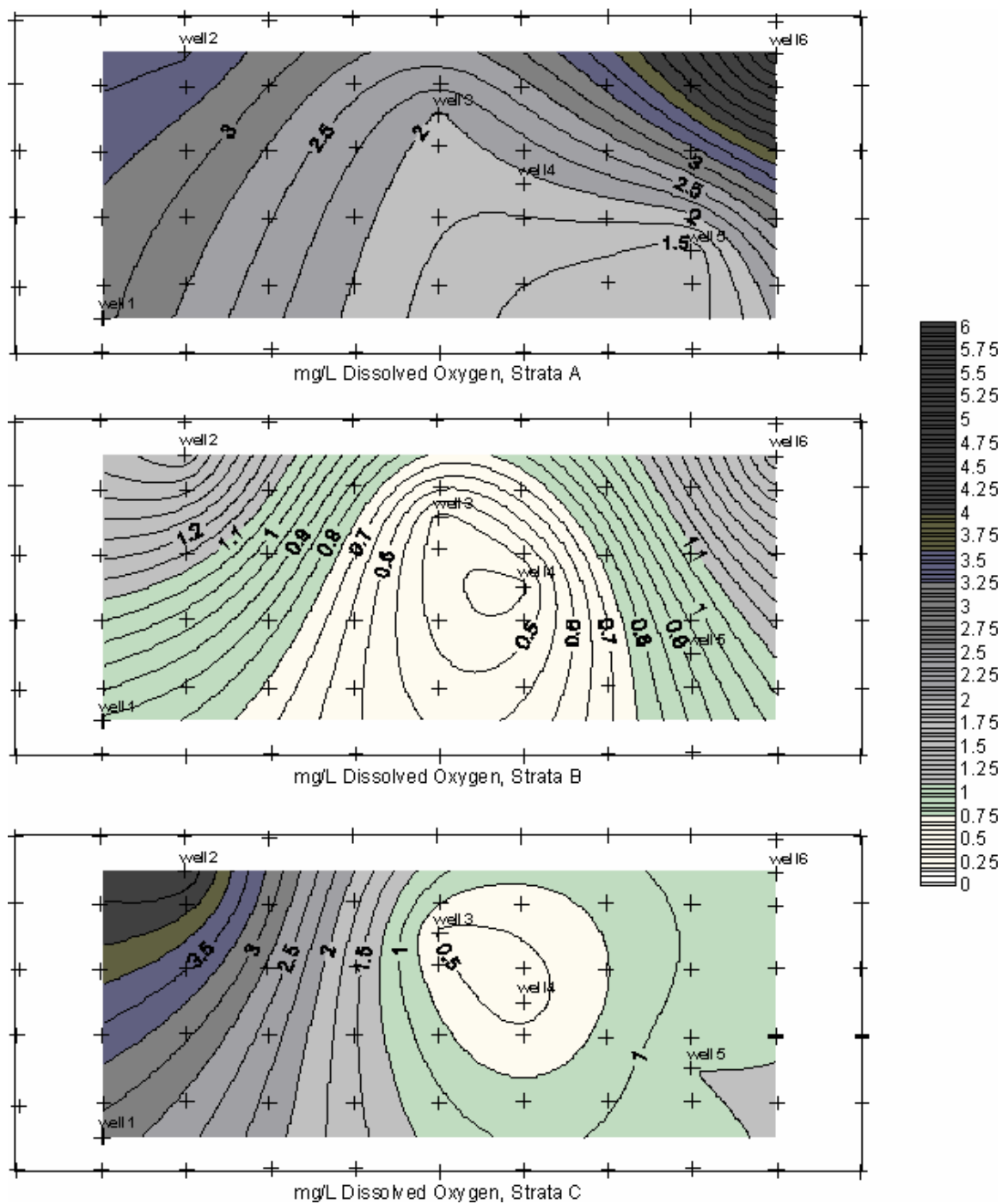
Appendix AG: Dissolved Oxygen, 8 January 2003

mg/L Dissolved Oxygen, Strata A

mg/L Dissolved Oxygen, Strata B

mg/L Dissolved Oxygen, Strata C
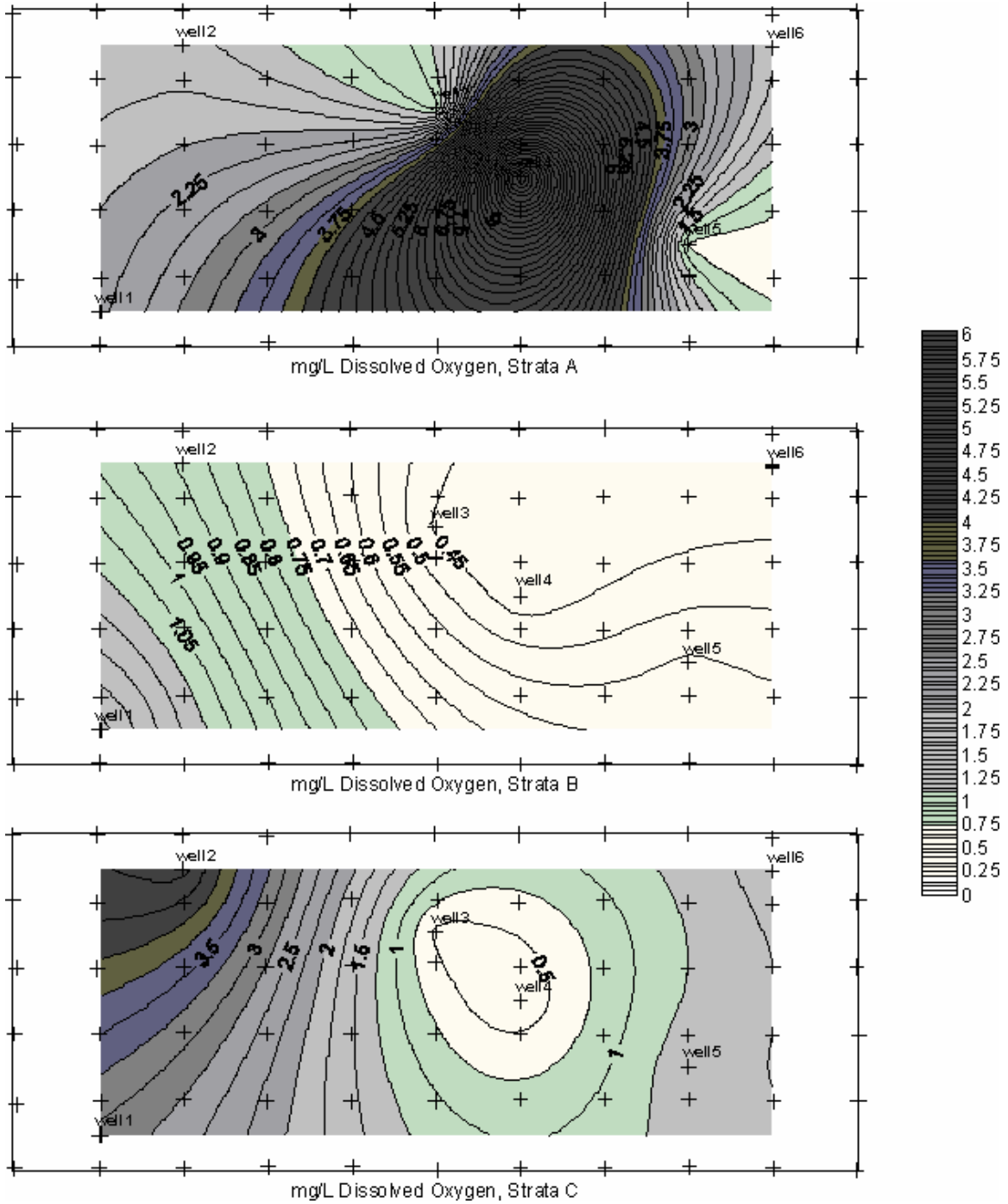
Appendix AH: Dissolved Oxygen, 9 January 2003

mg/L Dissolved Oxygen, Strata A

mg/L Dissolved Oxygen, Strata B

mg/L Dissolved Oxygen, Strata C
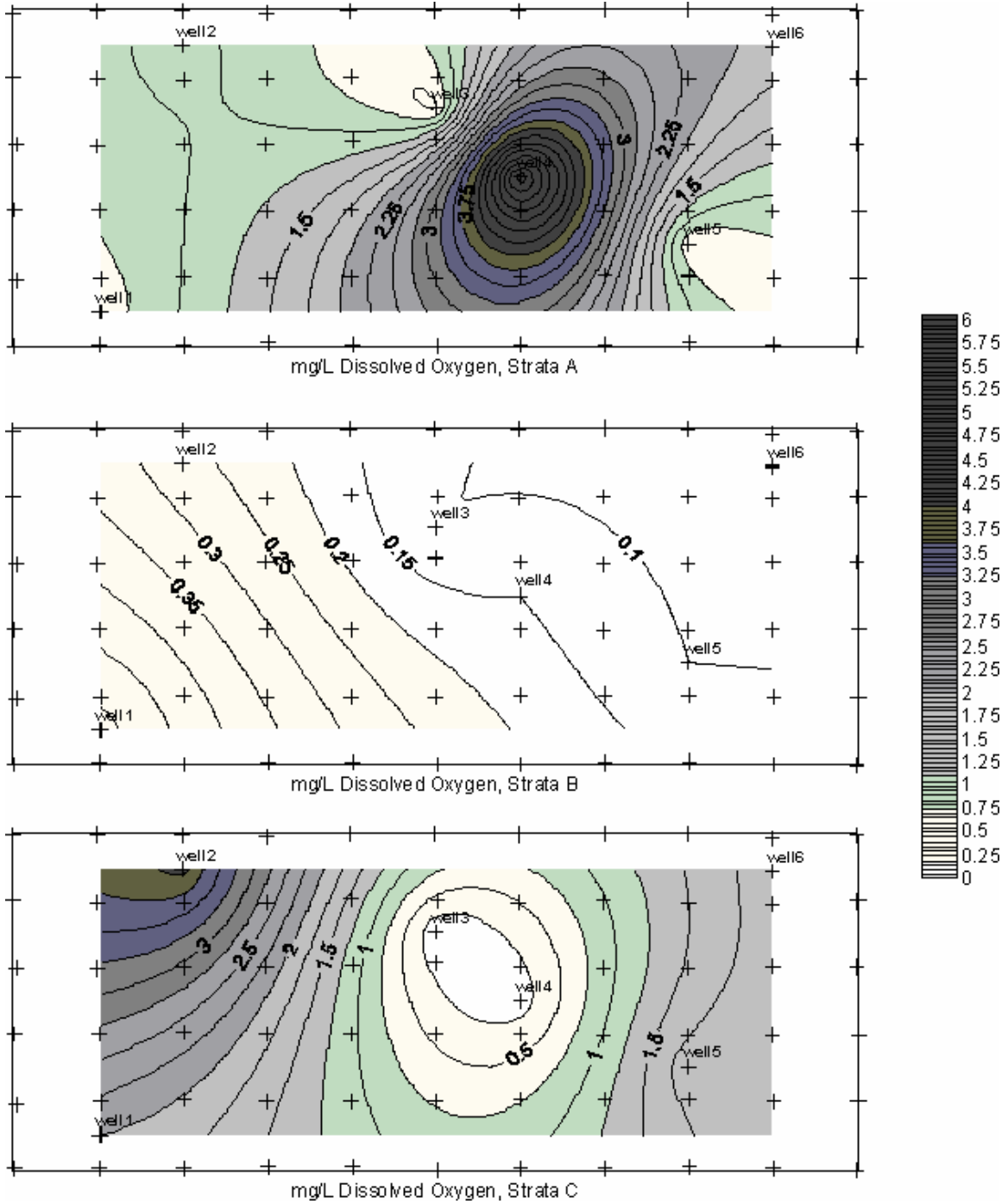
Appendix AI: Oxidation-Reduction Potential, 23 December 2002

ORP (mV), Strata A

ORP (mV), Strata B

ORP (mV), Strata C
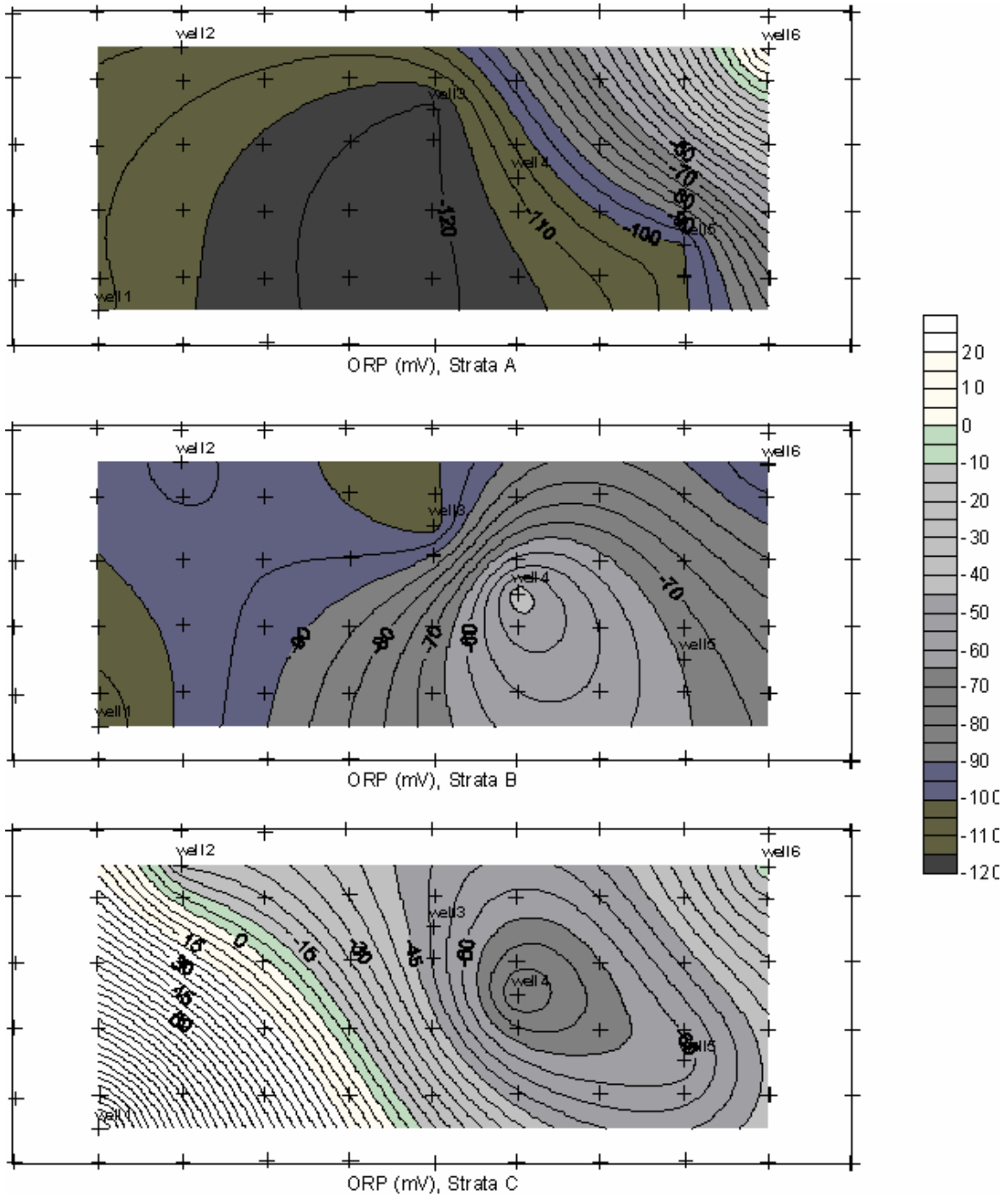
Appendix AJ: Oxidation-Reduction Potential, 8 January 2003

ORP (mV), Strata A

ORP (mV), Strata B

ORP (mV), Strata C
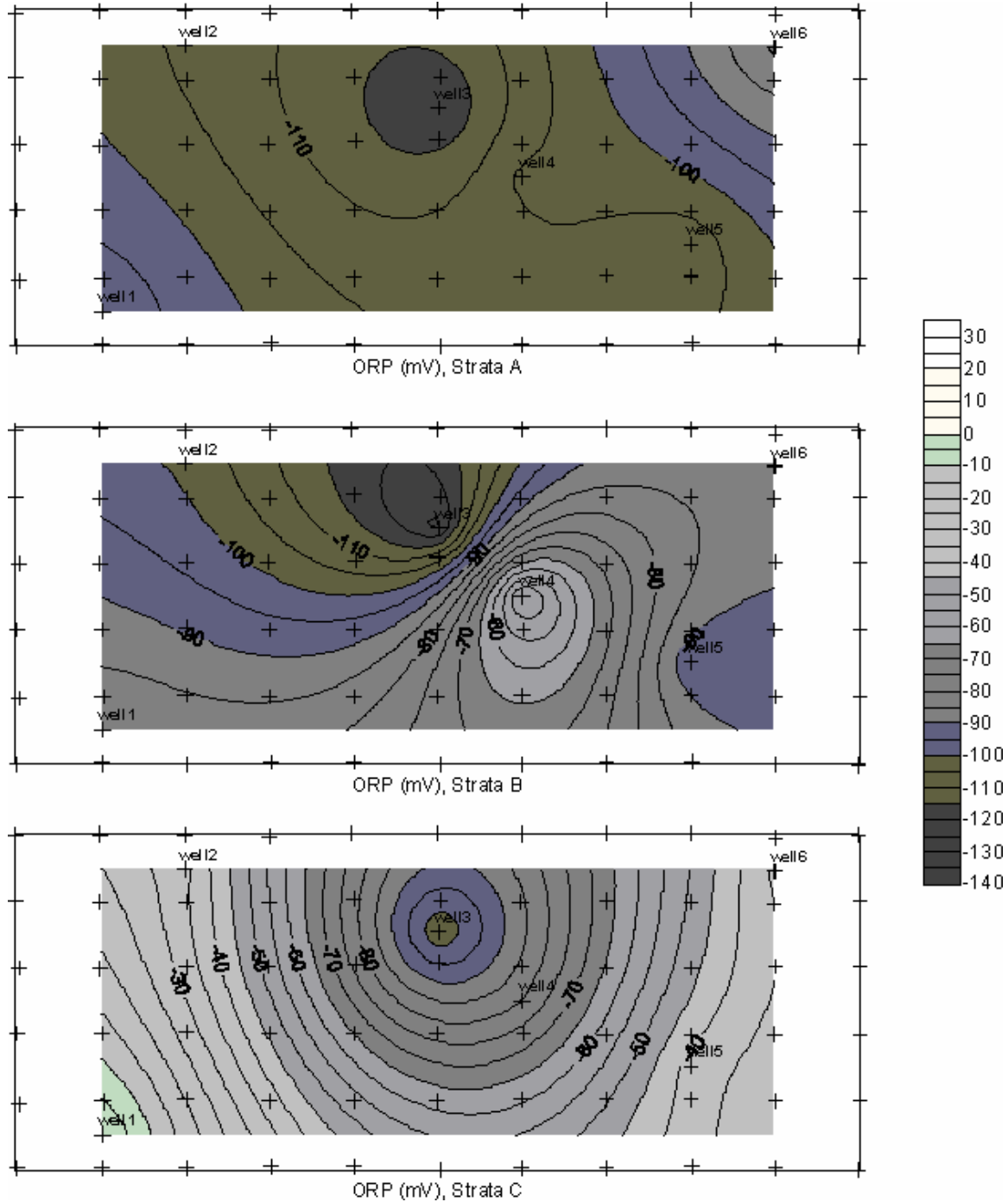
Appendix AK: Oxidation-Reduction Potential, 9 January 2003

ORP (mV), Strata A

ORP (mV), Strata B

ORP (mV), Strata C
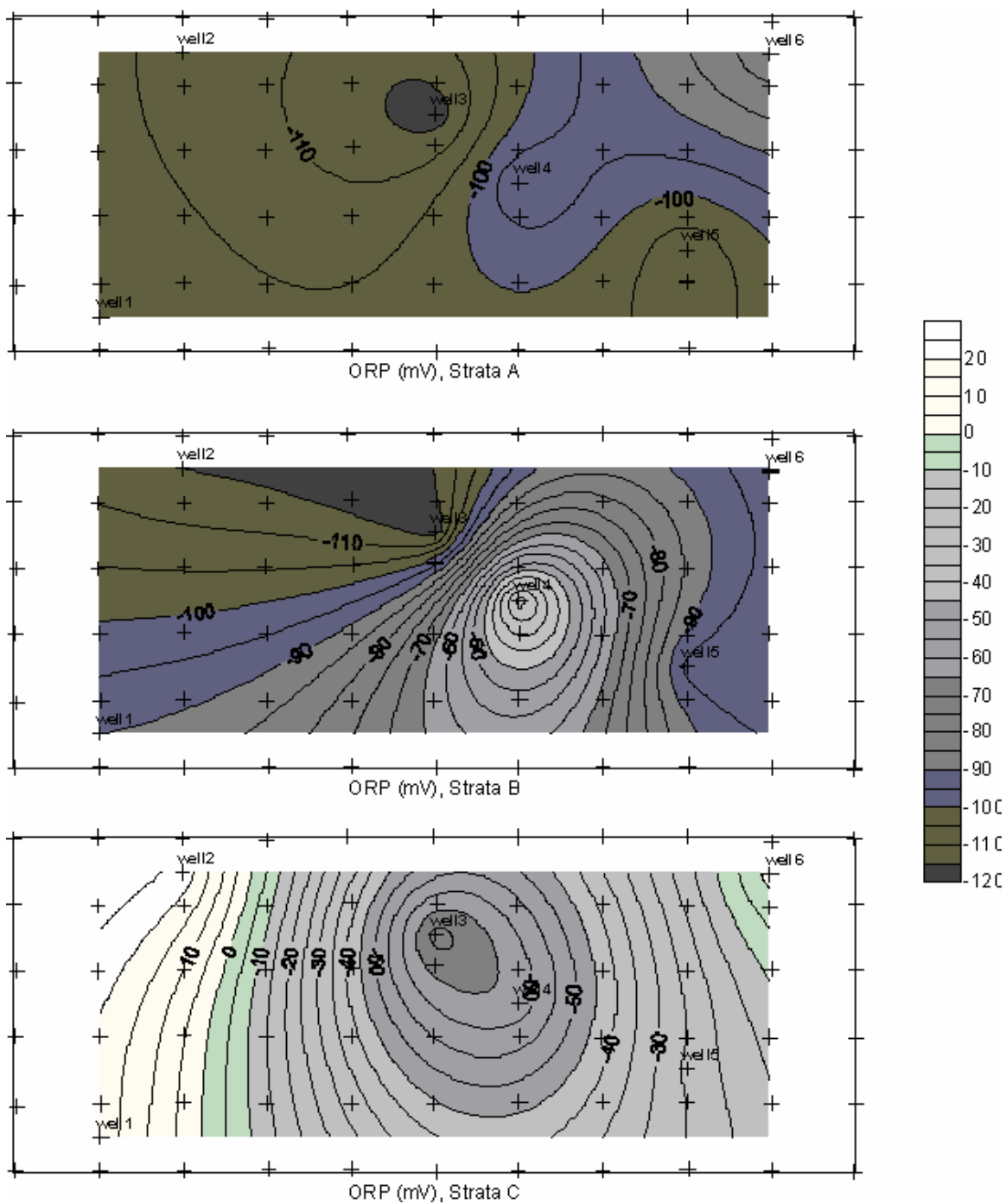
Appendix AL: pH, 23 December 2002

pH, Strata A

pH, Strata B

pH, Strata C
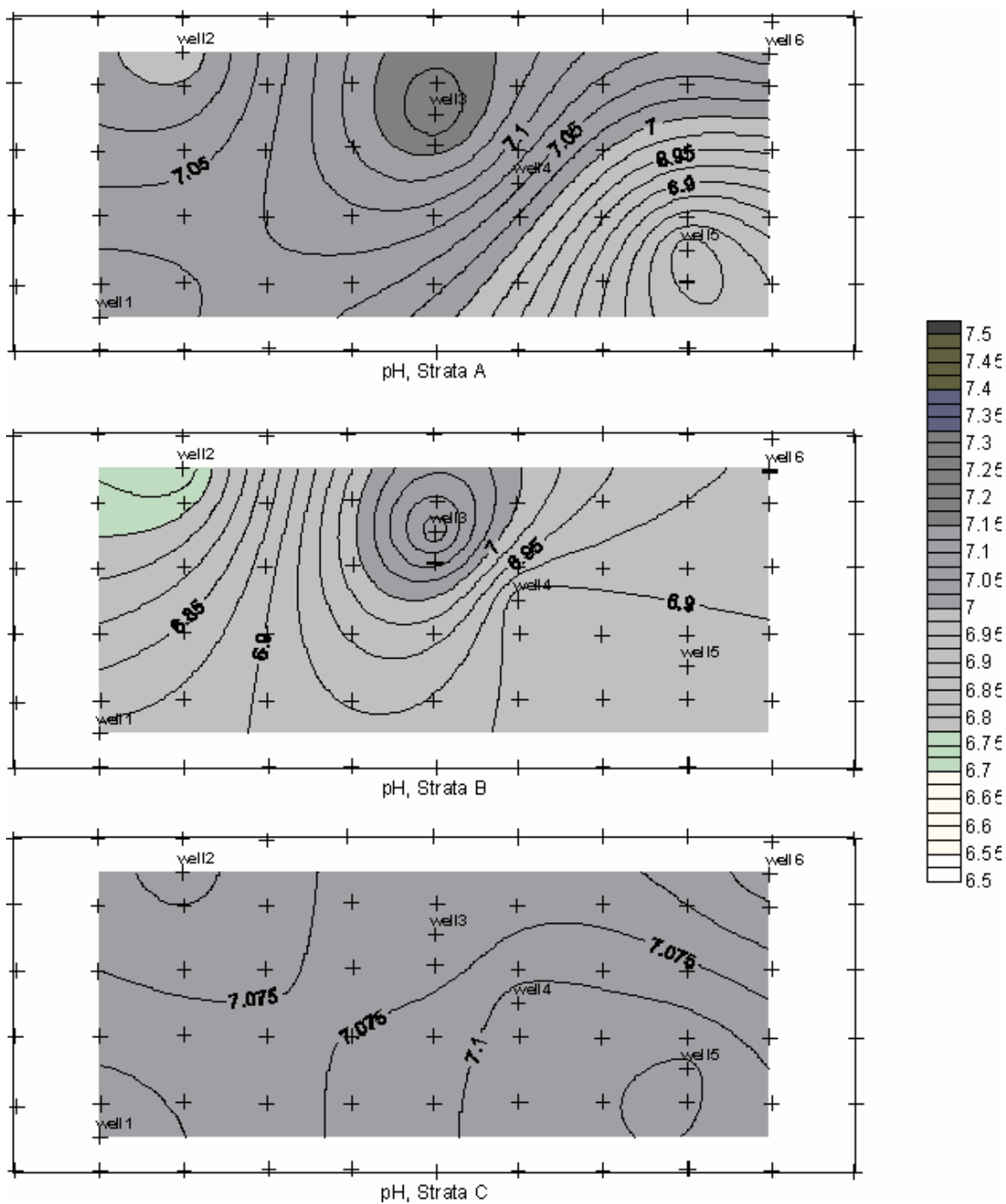
Appendix AM: pH, 8 January 2003

pH, Strata A

pH, Strata B

pH, Strata C
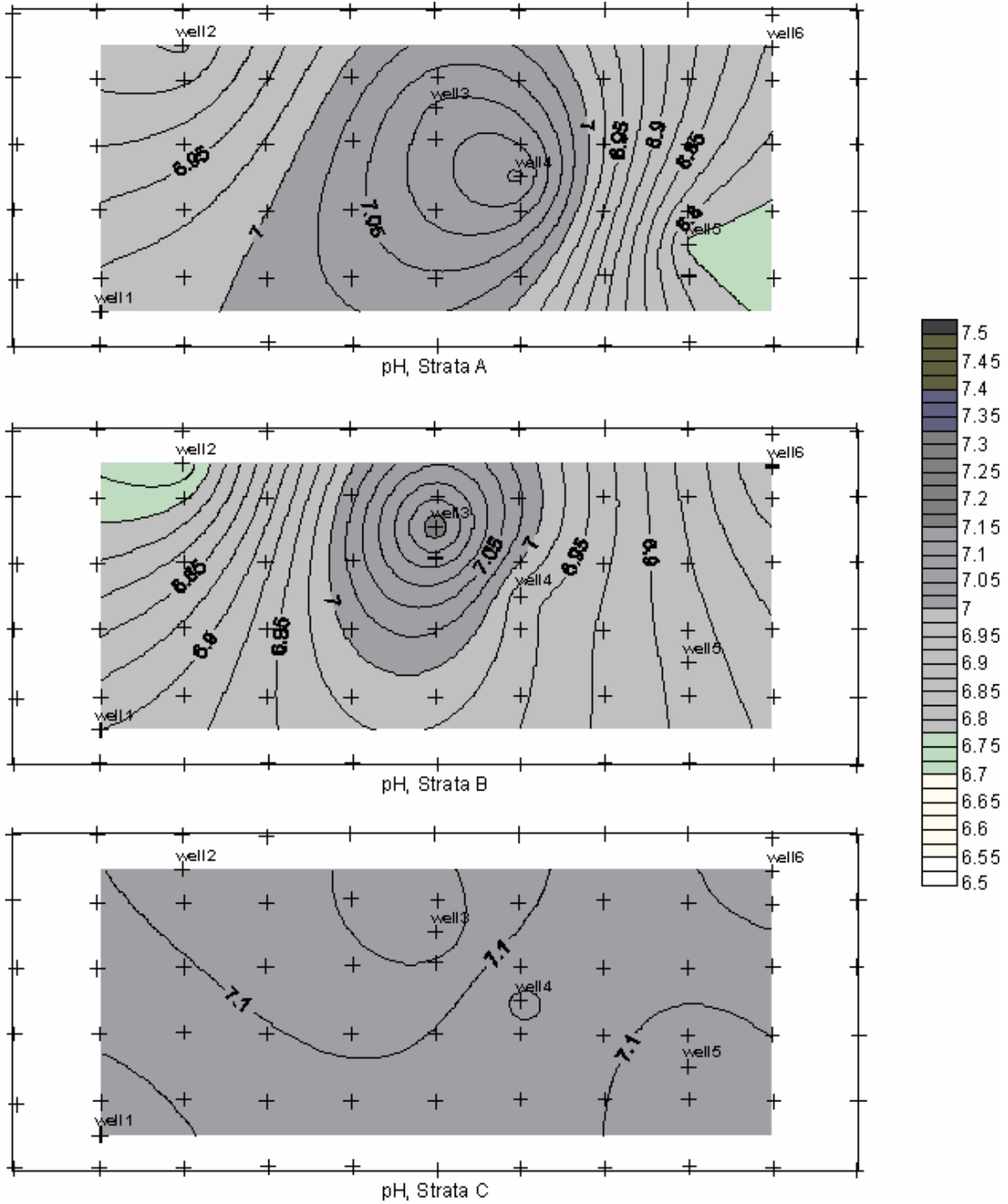
Appendix AN: pH, 9 January 2002

pH, Strata A

pH, Strata B

pH, Strata C
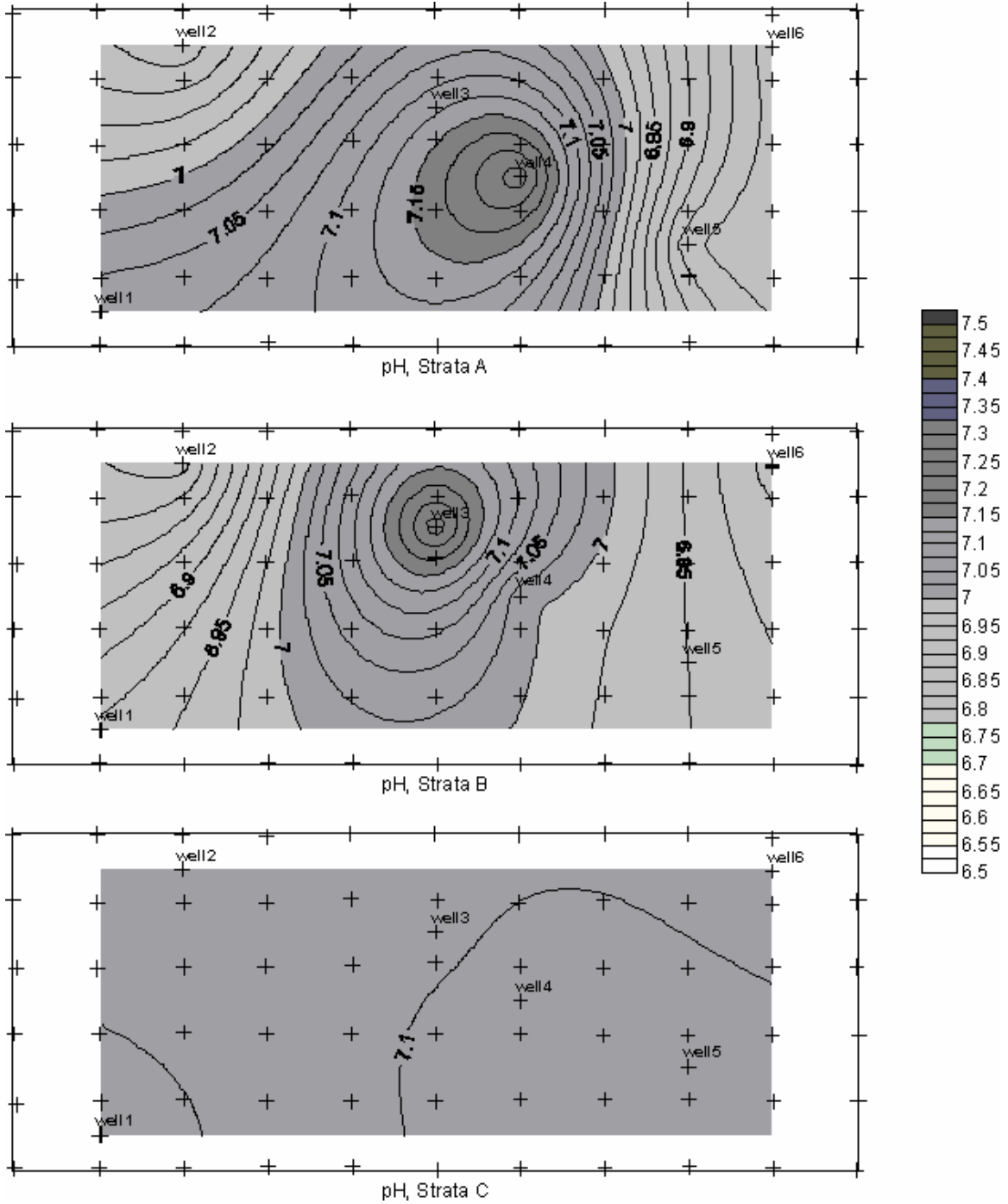
Appendix AO: Temperature °C, 23 December 2002

Temperature (C), Strata A

Temperature (C), Strata B

Temperature (C), Strata C
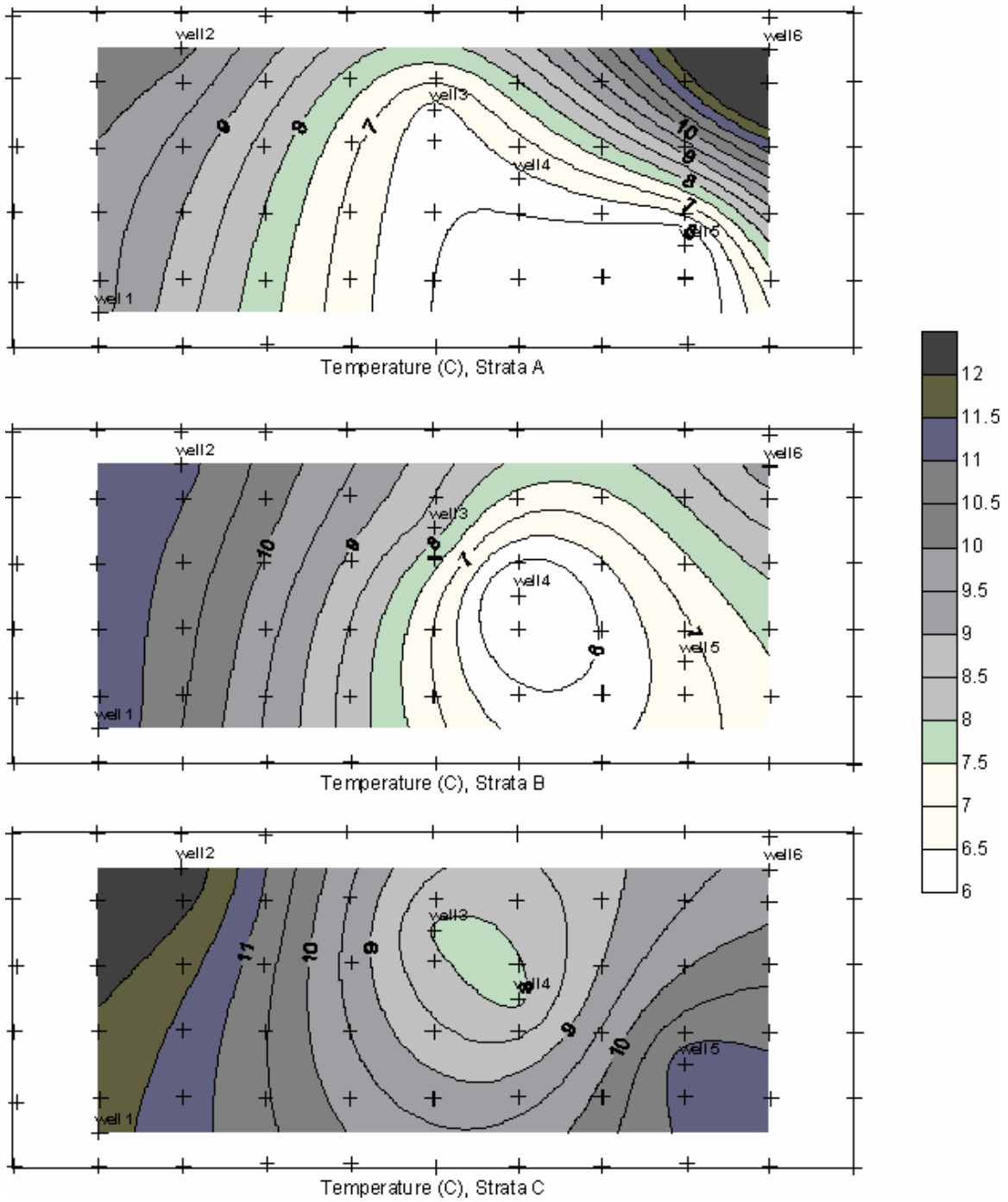
Appendix AP: Temperature °C, 8 January 2003

Temperature (C), Strata A

Temperature (C), Strata B

Temperature (C), Strata C
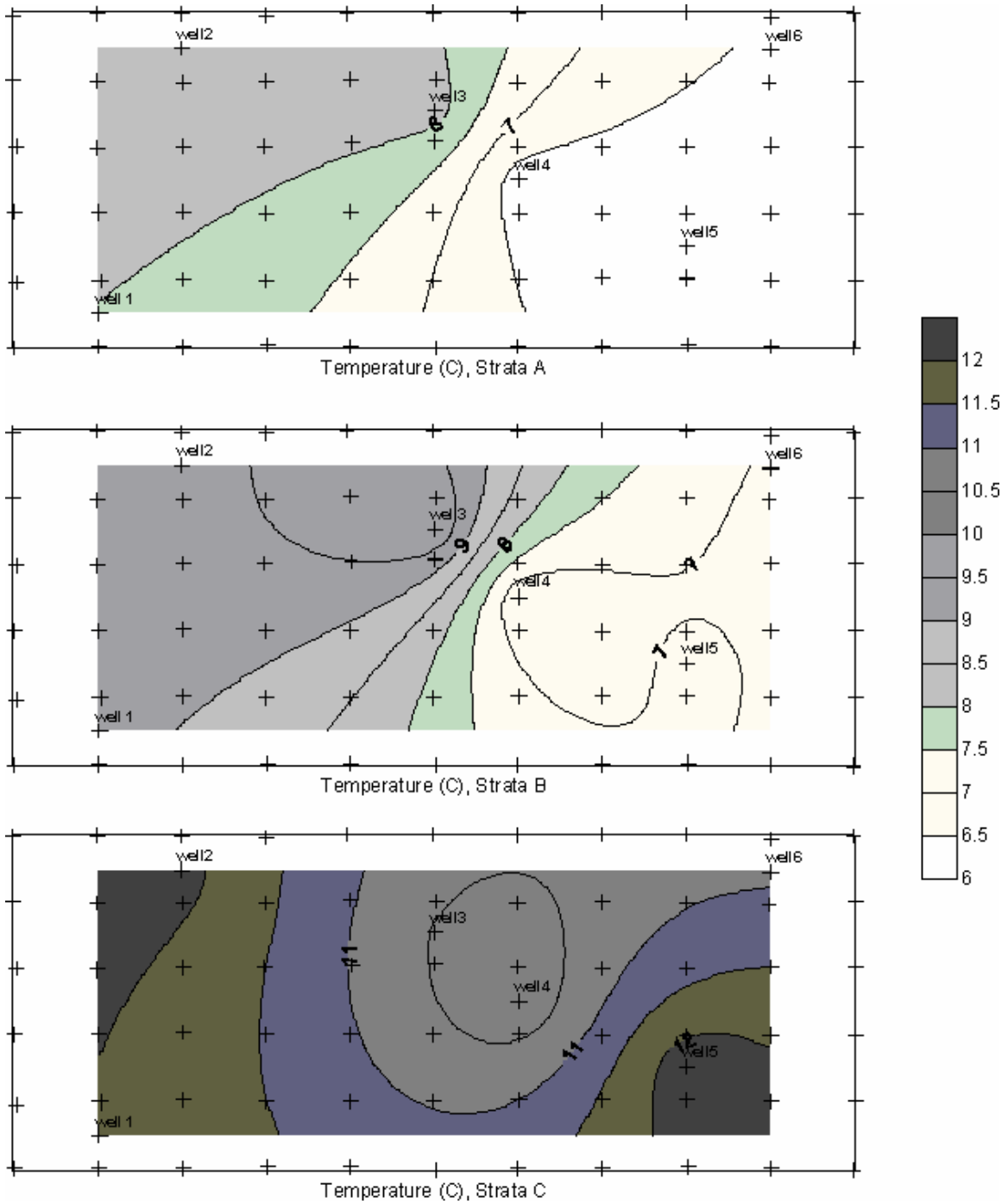
Appendix AQ: Temperature °C, 9 January 2003

Temperature (C), Strata A

Temperature (C), Strata B

Temperature (C), Strata C
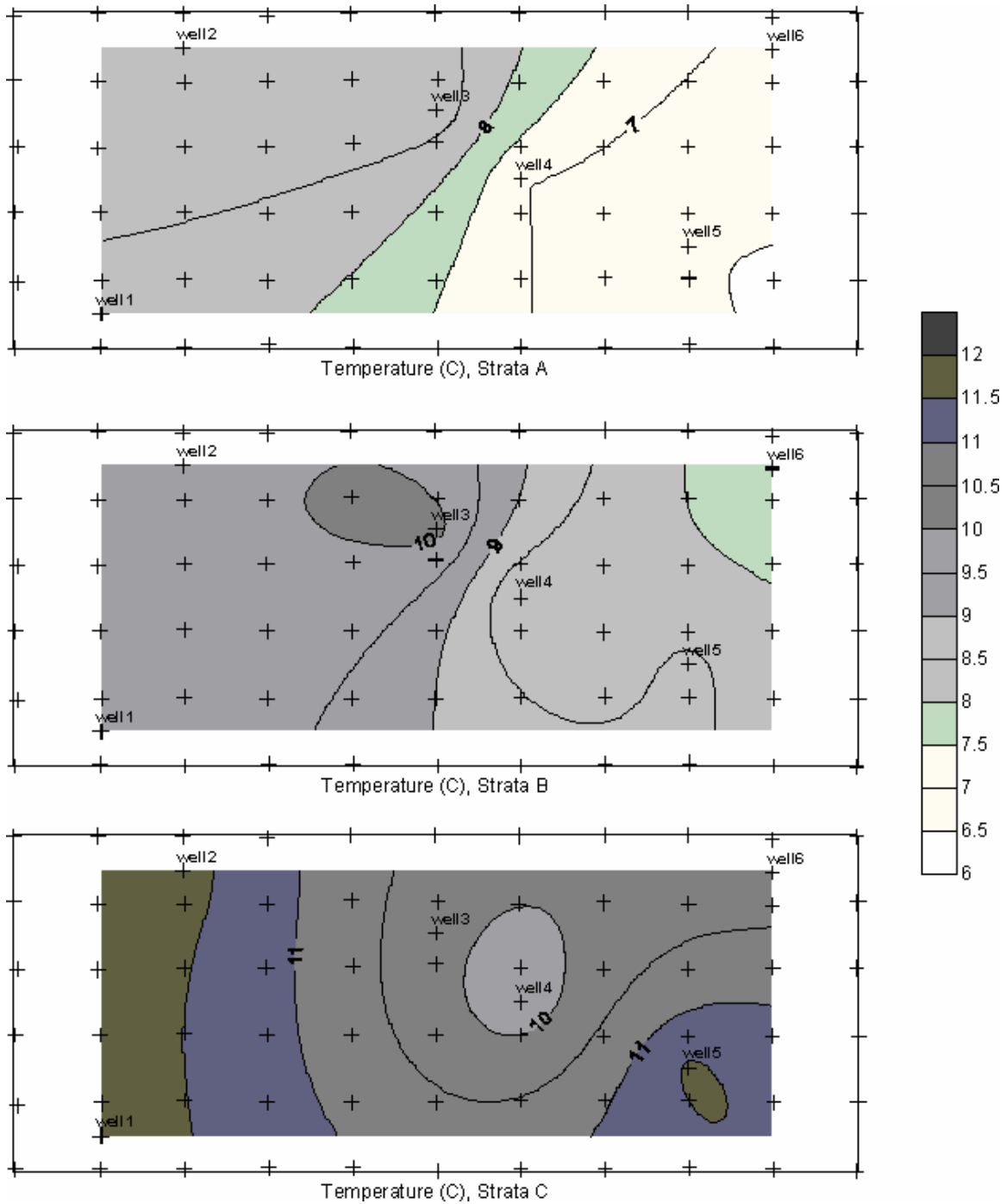
Appendix AR: Data Results: Strata A, January 2002 (Average)

Nest #	ppb Fluoride Jan 02	ppb Lactate Jan 02	ppb Acetate Jan 02	ppb Propionate Jan 02	ppb Formate Jan 02	ppb Butyrate Jan 02
1	99.81	22.46	60.79	0.00	2262.38	0.39
2	121.44	49.53	81.73	5.43	3039.71	0.21
3	*550.90	*5087.19	1182.52	*60.43	10531.42	*13.07
4	394.92	*5819.08	123.93	19.67	3893.19	0.00
5	261.16	4166.80	450.92	*195.92	8637.07	0.00
6	171.51	775.24	1153.45	33.13	3879.85	0.00
7		2183.69	260.35	3.48	8008.33	0.00
8	*568.10	650.53	239.07	0.30	3987.75	0.00
9	114.88	4818.95	222.37	0.31	3658.86	0.00
10	102.61	1322.03	3.62	0.31	909.14	0.00
11	299.46	2343.47	817.22	15.85	9425.45	0.00
12	186.23	2799.66	853.97	3.51	5689.22	0.00
13	*609.87	851.09	*8465.11	*426.11	10412.52	*69.60
14	147.12	666.45	4071.67	73.42	9663.50	0.00
15	163.39	1537.33	2226.41	34.88	8632.15	0.00
16	139.48	731.17	19.14	0.32	473.29	0.00
17	132.05	69.86	1079.60	0.27	10315.94	0.00
18	165.10	2478.60	153.88	47.24	2864.50	0.00
19	204.19	96.15	*3117.00	*125.43	10299.01	*35.24
20	*0.19	2.47	0.04	0.00	3.35	0.00
21	207.43	936.38	724.74	1.13	4564.20	1.88
22	106.51	41.59	18.44	0.30	1305.46	0.31
23	224.11	855.92	847.35	0.19	8561.31	0.00
24	235.07	2751.92	356.25	0.64	10155.93	0.84
25	284.90	2847.88	70.00	0.16	3538.34	0.00
26	210.45	476.22	868.96	0.00	8845.41	*23.56
27	177.01	3214.10	1795.22	30.90	11364.16	0.00
28	184.00	2259.28	796.19	1.64	11964.77	0.00
29	158.82	552.78	4.03	0.79	421.24	0.55
30	245.18	280.66	26.99	0.00	0.00	0.00
31	180.88	4540.32	453.63	5.44	10423.10	0.00
32	173.53	3819.63	98.24	0.00	3817.77	*2.97
33	238.18	*5108.81	232.44	0.00	3740.17	0.76
34	186.94	580.02	78.17	0.14	6933.59	0.00
35	195.55	2116.45	6.87	0.13	3135.78	0.00
36	177.52	1627.28	231.21	0.93	1913.56	0.00
37	214.87	*5632.01	20.16	16.90	2093.01	0.50
38	274.75	1687.42	816.94	1.92	7324.99	0.00
39	255.76	1242.68	411.48	0.00	8336.58	0.00
40	0.19	2.47	0.04	0.00	3.35	0.00
41	101.63	1382.36	131.68	0.19	10637.68	0.34
42	177.12	625.33	115.26	0.00	10998.55	*1.45
43	182.43	566.94	879.27	4.96	6393.21	*2.65
44	222.93	892.42	736.48	2.20	9726.18	1.03

Nest #	ppb Fluoride Jan 02	ppb Lactate Jan 02	ppb Acetate Jan 02	ppb Propionate Jan 02	ppb Formate Jan 02	ppb Butyrate Jan 02
45	176.94	1279.15	15.71	0.15	2367.24	0.00
46	175.50	446.04	188.65	0.00	5963.59	0.00
47	230.34	926.21	*1268.73	511.98	8025.34	*12.22
48	137.43	710.56	51.29	0.00	1700.72	0.00
49	222.61	1566.17	*314.26	58.33	9657.81	0.00
50	199.29	1027.08	28.23	0.18	9810.34	0.00
51	249.68	2146.14	106.15	0.20	8917.57	0.00
52	241.72	1382.15	60.00	0.00	4775.23	0.00
53	231.56	1215.07	48.41	0.22	5622.95	*34.56
54	159.04	3579.47	1346.21	0.20	25701.02	0.00
55	196.15	610.30	959.12	0.75	13821.41	0.00
56	87.49	2442.25	4516.71	0.43	4376.13	0.00
57	181.04	2056.52	1618.13	0.54	10658.34	0.00
58	177.99	351.55	1251.41	0.66	14595.41	0.00
59	180.34	3087.53	*5698.77	26.89	15981.66	0.00
60	*0.19	2.47	0.04	*0.00	3.35	0.00
61	113.57	1369.07	*8862.60	92.37	*26555.85	0.00
62	109.15	41.32	*31992.09	*607.24	*32582.99	*36.57
63	182.04	742.47	*14188.30	*355.25	*79702.12	*35.71
64	162.39	277.38	*5135.69	13.59	14942.95	0.00
65	170.75	214.53	271.46	2.10	5104.06	0.00
66	160.76	735.23	*5133.52	22.43	*49707.93	0.30

Nest #	ppm Chloride Jan 02	ppb Nitrite Jan 02	ppb Bromide Jan 02	ppm Nitrate Jan 02	ppm Sulfate Jan 02
1	77.51	2.32	0.27	0.00	38.21
2	76.00	2.14	1.61	0.00	20.94
3	61.25	2.06	3.32	0.00	8.03
4	83.94	1.91	0.60	0.00	2.52
5	54.90	0.07	3.24	0.00	6.51
6	*143.71	1.00	2.38	0.00	6.54
7	69.85	2.00	1.49	0.00	5.73
8	62.91	3.05	3.55	0.00	7.89
9	69.92	0.57	1.65	0.00	3.09
10	73.47	*7.26	0.09	0.00	38.52
11	52.69	0.86	3.04	0.00	5.13
12	55.69	0.55	4.01	0.00	5.04
13	96.43	0.72	3.35	0.00	5.73
14	66.87	1.23	4.56	0.00	4.69
15	80.88	0.56	2.79	0.00	5.50
16	80.73	*10.28	0.10	0.00	*51.62
17	45.92	1.02	3.59	0.00	1.72
18	82.84	0.89	2.94	0.00	4.01
19	61.75	0.60	3.14	0.00	6.70
20	81.12	0.01	0.00	0.00	42.50
21	74.59	2.74	0.98	0.00	13.63
22	79.30	6.06	0.10	0.00	25.89
23	61.80	0.38	1.89	0.00	4.42

Nest #	ppm Chloride Jan 02	ppb Nitrite Jan 02	ppb Bromide Jan 02	ppm Nitrate Jan 02	ppm Sulfate Jan 02
24	77.61	3.12	3.51	0.00	6.88
25	76.04	4.40	0.73	0.00	35.15
26	244.01	1.00	3.60	0.00	23.80
27	55.47	0.93	2.92	0.00	2.26
28	63.57	1.45	5.31	0.00	7.61
29	80.93	4.82	0.09	*1.82	*50.86
30	81.94	*18.63	0.08	*1.80	*50.59
31	61.13	1.17	2.82	0.00	6.54
32	69.39	1.04	3.02	0.00	10.08
33	81.56	4.71	0.48	0.00	19.95
34	55.28	6.46	2.58	0.00	5.29
35	77.94	1.38	0.17	0.00	7.77
36	77.59	*14.73	0.34	0.00	14.63
37	79.63	0.38	0.39	0.00	*46.70
38	62.60	2.08	2.47	0.00	3.07
39	65.40	0.28	3.31	0.00	5.32
40	65.58	0.01	0.00	0.00	5.59
41	82.33	*8.54	2.81	0.00	6.10
42	68.98	0.36	2.74	0.00	2.15
43	81.16	0.24	2.23	0.00	1.81
44	53.12	*9.31	4.15	0.00	8.77
45	*157.15	4.25	2.46	0.00	*47.55
46	61.75	1.48	3.99	0.00	1.83
47	41.76	*7.53	3.92	0.00	6.04
48	*165.10	1.20	2.41	0.00	33.25
49	79.16	0.62	3.22	0.00	3.47
50	*270.23	1.08	2.65	0.00	21.09
51	66.51	3.79	2.94	0.00	6.91
52	78.43	0.37	0.47	0.00	5.72
53	80.23	0.47	0.17	0.00	16.24
54	58.93	0.32	3.66	0.00	7.76
55	73.95	0.55	1.85	0.00	12.77
56	*382.52	1.70	2.46	0.00	*163.31
57	79.94	0.27	1.05	0.00	6.12
58	78.99	0.23	0.41	0.00	4.23
59	53.10	0.12	2.78	0.00	6.32
60	52.29	0.01	0.00	0.00	6.03
61	49.64	0.44	2.32	0.00	5.04
62	69.01	1.00	2.62	0.00	4.19
63	*131.67	5.32	2.92	0.00	*47.92
64	70.99	0.80	1.47	0.00	7.60
65	81.38	0.64	0.14	0.00	22.63
66	77.21	0.14	1.11	0.00	5.29

Appendix AS: Data Results: Strata B, Jan 02 (Average)

Nest #	ppb Fluoride Jan 02	ppb Lactate Jan 02	ppb Acetate Jan 02	ppb Propionate Jan 02	ppb Formate Jan 02	ppb Butyrate Jan 02
1	126.65	17.45	*2790.60	*121.30	7848.40	*486.57
2	144.60	41.30	313.00	0.00	11204.80	0.20
3		*6779.53	85.33	*56.67	5322.43	*2.60
4	*451.80	*7779.43	23.17	0.00	3571.83	*14.97
5	264.88	4166.58	289.80	0.23	11641.63	0.00
6	216.70	1052.83	186.80	0.00	7932.13	0.00
7		1641.00	97.90	0.00	9531.77	0.00
8		882.73	157.83	0.00	7746.23	0.00
9	147.03	*6425.93	9.30	0.00	2692.17	0.00
10	108.43	1721.20	1.80	0.70	0.00	0.00
11	365.07	3116.53	135.93	8.03	7764.27	0.00
12	181.13	2791.40	644.80	0.17	11850.07	0.63
13		1679.30	999.30	3.90	11229.70	0.00
14	121.17	877.47	850.37	0.37	9934.93	*11.20
15	109.87	2029.20	356.33	2.17	10112.33	0.00
16	155.47	708.33	188.47	0.23	6574.70	0.00
17	46.63	13.13	281.97	0.57	12413.70	0.00
18	110.90	2466.03	909.30	*91.67	15475.80	0.00
19	120.03	102.63	758.73	*24.87	14176.93	0.17
20	0.19	2.47	0.04	0.00	3.35	0.00
21	216.80	938.43	54.03	0.00	6711.50	0.00
22	101.93	13.83	3.67	0.00	756.03	0.00
23	242.37	882.27	469.83	0.00	9532.47	0.00
24	235.67	2754.90	1047.43	3.40	14395.93	0.00
25	317.53	2762.43	411.93	0.00	15318.07	0.00
26	293.93	630.57	158.47	2.80	6921.53	*5.23
27	246.77	4240.47	508.00	0.20	13304.43	0.00
28	299.50	2184.90	618.37	4.30	11518.03	0.00
29	173.70	518.73	14.20	0.00	1452.90	0.00
30	270.67	263.97	1048.60	0.00	10377.23	0.00
31	164.13	4557.20	*1813.63	*196.73	15671.50	*163.03
32	244.30	5084.93	558.37	*32.67	9772.27	0.00
33	241.43		184.10	0.77	5074.13	0.00
34	193.83	501.70	1036.03	3.43	7854.33	0.20
35	186.80	2088.93	888.50	*24.80	3403.93	0.00
36	214.27	2126.27	873.83	0.63	9076.20	0.00
37	240.97	*5622.97	222.47	6.70	9531.63	0.33
38	296.13	1706.73	49.77	0.33	9008.50	0.00
39	266.33	1710.97	868.47	4.13	11269.23	0.00
40	0.19	2.47	0.04	0.00	3.35	0.00
41	191.50	1376.40	197.03	0.57	10829.57	0.00
42	254.47	795.00	515.87	0.20	9553.10	0.00
43	200.63	573.10	262.33	0.30	13099.77	0.00
44	237.70	867.93	162.47	0.00	6239.20	0.00

Nest #	ppb Fluoride Jan 02	ppb Lactate Jan 02	ppb Acetate Jan 02	ppb Propionate Jan 02	ppb Formate Jan 02	ppb Butyrate Jan 02
45	199.37	1290.03	386.73	*89.73	5919.97	*9.40
46	113.60	624.23	576.07	1.07	12623.97	0.00
47	274.80	1250.03	164.90	0.17	8348.87	0.00
48	197.63	693.37	66.83	0.57	2638.10	0.00
49	213.90	1538.67	1768.23	*77.10	10406.17	0.00
50	206.50	1095.10	432.27	*13.07	10114.30	0.43
51	239.70	2182.57	320.73	0.00	5764.37	0.00
52	248.80	1816.27	157.70	0.13	5462.27	0.00
53	270.77	1556.40	84.77	0.33	5054.43	0.00
54	226.77	3569.57	1084.77	1.73	11935.93	0.00
55	177.03	553.10	687.63	0.03	11126.53	0.00
56	133.50	3214.33	65.10	0.00	3315.20	0.00
57	188.70	2066.13	131.03	0.00	6758.17	0.00
58	173.63	358.57	25.63	0.00	3216.70	0.00
59	197.53	3079.90	322.80	*28.00	10522.43	0.00
60	0.19	2.47	0.04	0.00	3.35	0.00
61	144.53	1311.30	1044.43	1.70	13832.83	0.00
62	154.60	60.93	549.80	0.17	11939.17	0.00
63	87.27	437.87	1418.37	1.10	14150.53	0.00
64	115.30	243.07	645.20	0.30	9330.00	*32.57
65	143.57	251.73	307.00	0.23	5912.47	0.00
66	130.80	947.77	1634.13	6.13	14088.47	0.00

Nest #	ppm Chloride Jan 02	ppb Nitrite Jan 02	ppb Bromide Jan 02	ppb Nitrate Jan 02	ppm Sulfate Jan 02
1	65.85	*30.57	2.00	0.00	*26.39
2	49.86	12.17	3.77	0.00	*135.07
3	75.31	1.63	1.17	0.00	*23.80
4	80.86	0.73	0.70	0.00	*23.72
5	56.03	7.33	3.37	0.00	1.09
6	59.25	1.07	3.57	0.00	3.90
7	75.25	5.23	1.33	0.00	2.53
8	68.08	0.43	2.67	0.00	0.49
9	81.43	3.93	0.93	0.00	*36.69
10	82.09	6.90	0.10	*2.50	*51.16
11	48.96	11.73	3.23	0.00	0.70
12	66.03	0.40	3.23	0.00	0.20
13	57.34	0.00	3.80	0.00	0.24
14	70.95	4.27	3.80	0.00	0.41
15	73.29	0.73	4.27	0.00	0.53
16	80.10	0.53	1.50	0.00	6.61
17	47.98	0.33	3.40	0.00	0.24
18	56.34	0.10	3.73	0.00	0.13
19	59.11	0.60	3.37	0.00	0.51
20	79.95	0.01	0.00	*0.78	*39.13
21	78.21	1.73	0.70	0.00	9.76
22	81.53	2.00	0.43	0.00	*31.93
23	80.01	0.57	0.77	0.00	0.46

Nest #	ppm Chloride Jan 02	ppb Nitrite Jan 02	ppb Bromide Jan 02	ppb Nitrate Jan 02	ppm Sulfate Jan 02
24	53.25	1.50	3.50	0.00	0.12
25	68.21	8.47	2.10	0.00	15.54
26	51.58	2.10	3.37	0.00	2.25
27	55.70	0.90	3.50	0.00	0.20
28	75.51	9.37	2.77	0.00	2.03
29	82.99	0.73	0.53	0.00	*31.21
30	68.77	0.47	3.33	0.00	4.75
31	74.80	*29.77	3.20	0.00	11.67
32	66.30	2.13	2.67	0.00	3.57
33	81.10	1.97	0.63	0.00	*25.61
34	72.79		1.47	0.00	0.18
35	78.08	0.83	0.43	0.00	0.61
36	78.97	2.07	0.53	0.00	0.04
37	64.17	6.33	2.17	0.00	5.74
38	53.58	4.03	3.10	0.00	1.06
39	66.14	9.47	2.60	0.00	0.87
40	57.88	0.01	0.00	0.00	0.39
41	59.49	0.30	2.57	0.00	0.01
42	71.54	1.70	1.57	0.00	0.31
43	59.78	5.47	3.83	0.00	1.72
44	51.10	0.53	3.23	0.00	0.40
45	61.03	*36.13	2.73	0.00	0.83
46	52.11	0.63	3.47	0.00	0.01
47	40.98	1.70	3.83	0.00	0.13
48	66.20	4.30	1.87	0.00	*22.55
49	109.02	4.77	3.57	0.00	2.83
50	51.94	9.80	3.80	0.00	0.73
51	78.16	5.20	0.70	0.00	3.22
52	73.60	9.73	0.83	0.00	5.16
53	78.68	5.70	0.63	0.00	0.77
54	69.97	7.00	3.10	0.00	0.12
55	46.10	9.83	3.27	0.00	5.61
56	44.32	0.80	3.15	0.00	5.35
57	75.02	2.60	0.90	0.00	0.53
58	77.54	7.10	0.30	0.00	10.59
59	47.66	1.13	3.63	0.00	1.12
60	67.22	0.01	0.00	0.00	0.14
61	39.88	1.30	4.07	0.00	0.37
62	37.24		4.20	0.00	0.10
63	40.75	2.97	4.20	0.00	0.20
64	72.36	1.63	1.10	0.00	1.34
65	79.26	0.37	0.37	0.00	0.37
66	71.02		1.13	0.00	0.37

Appendix AT: Data Results: Strata C, Jan 02 (Average)

Nest #	ppb Fluoride Jan 02	ppb Lactate Jan 02	ppb Acetate Jan 02	ppb Propionate Jan 02	ppb Formate Jan 02	ppb Butyrate Jan 02
1	146.55	4.94	3.19	0.13	0.00	0.00
2	143.02	7.97	0.00	0.00	0.00	0.00
3	146.57	8.04	0.00	0.00	0.00	0.00
4	155.34	16.42	0.00	0.00	0.00	0.00
5	157.66	11.91	0.69	0.00	*1657.43	0.00
6	unavailable	unavailable	unavailable	unavailable	unavailable	unavailable
7	146.27	33.91	2.98	0.59	0.00	0.00
8	152.01	13.24	0.85	0.24	0.00	*0.49
9	174.51	113.76	*22.99	0.46	493.94	0.00
10	*104.04	10.35	0.36	0.19	0.00	0.00
11	*98.33	10.16	3.28	0.18	0.00	0.00
12	143.85	18.22	0.52	0.38	0.00	0.00
13	*101.56	14.47	0.16	*2.69	0.00	0.00
14	*107.45	7.12	1.02	0.00	0.00	0.00
15	155.36	17.41	0.17	0.29	0.00	0.00
16	156.94	8.39	1.52	0.97	0.00	0.00
17	151.75	11.58	0.00	0.27	0.00	0.00
18	152.11	14.66	0.32	0.00	0.00	0.00
19	146.28	24.07	*10.29	0.20	0.00	0.00
20	*0.19	2.47	0.04	0.00	3.35	0.00
21	163.26	*92.00	0.61	0.83	0.00	0.00
22	*105.57	10.29	2.96	0.71	326.11	0.00
23	*110.54	5.59	0.94	1.36	357.93	0.00
24	*114.94	6.91	5.59	0.38	448.29	0.00
25	130.70	2.83	0.37	1.05	0.00	0.00
26	158.37	16.89	0.19	0.63	0.00	0.00
27	*189.14	*136.17	*15.62	0.00	471.17	*7.17
28	159.28	5.66	0.45	0.92	319.67	*580.42
29	*194.98	33.15	*134.17	1.02	*5166.27	*0.36
30	164.52	11.70	3.25	0.92	174.99	*3.95
31	158.68	43.41	0.70	1.12	0.00	*51.17
32	154.71	8.76	0.17	0.60	0.00	0.00
33	156.48	12.24	6.55	0.15	*2367.09	0.00
34	154.63	5.74	0.45	0.75	346.24	0.00
35	159.74	5.33	1.26	0.48	427.42	*0.87
36	158.82	10.17	*9.14	0.50	521.94	0.00
37	150.31	5.43	1.76	0.45	460.20	*0.50
38	169.55	*65.47	2.15	0.69	331.91	*75.42
39	153.36	10.20	0.09	0.26	0.00	0.00
40	*0.19	2.47	0.04	0.00	3.35	0.00
41	157.27	15.54	0.47	0.63	0.00	0.00
42	152.95	14.61	0.12	0.17	166.82	*43.85
43	151.85	9.15	3.47	0.36	*1531.21	0.00
44	157.41	15.29	1.01	0.42	0.00	0.00

Nest #	ppb Fluoride Jan 02	ppb Lactate Jan 02	ppb Acetate Jan 02	ppb Propionate Jan 02	ppb Formate Jan 02	ppb Butyrate Jan 02
45	158.85	14.69	0.12	0.00	235.26	0.00
46	153.21	26.02	0.13	0.00	482.28	0.00
47	152.40	19.51	0.00	0.51	121.42	*0.63
48	149.87	4.66	0.53	0.90	245.55	0.00
49	159.27	*288.97	*8.87	0.31	931.77	0.00
50	156.51	7.74	0.50	0.34	347.27	0.00
51	156.79	4.87	0.11	0.55	555.86	*4.95
52	159.07	6.44	1.01	0.00	0.00	*67.57
53	171.96	29.49	2.15	0.00	740.48	0.00
54	166.98	42.09	5.95	0.55	1105.50	*1.17
55	148.85	26.22	0.31	0.34	0.00	0.00
56	154.09	9.25	0.21	0.14	0.00	0.00
57	156.06	9.05	0.53	0.54	10.21	0.00
58	160.26	22.44	*34.37	*5.30	551.86	*26.40
59	156.07	13.82	0.28	0.40	588.93	0.00
60	*0.19	2.47	0.04	0.00	3.35	0.00
61	150.03	18.13	0.11	0.00	136.56	0.00
62	157.03	*151.36	0.00	0.00	1150.04	0.00
63	150.96	22.22	0.09	0.00	605.80	0.00
64	159.63	19.72	1.51	0.33	348.91	0.00
65	148.97	23.37	0.72	0.00	1114.50	0.00
66	156.07	*111.03	0.09	0.13	136.04	0.00

Nest #	ppm Chloride Jan 02	ppb Nitrite Jan 02	ppb Bromide Jan 02	ppb Nitrate Jan 02	ppm Sulfate Jan 02
1	82.08	3.57	0.03	2.81	49.68
2	81.77	12.23	0.05	1.54	48.97
3	81.33	*17.16	0.04	1.40	49.52
4	81.01	*34.06	0.04	1.60	50.68
5	81.88	3.77	0.05	1.71	49.77
6	unavailable	unavailable	unavailable	unavailable	unavailable
7	82.07	8.04	0.04	2.28	49.50
8	83.12	1.29	0.04	2.35	49.59
9	85.31	16.23	0.04	2.48	51.40
10	80.78	*32.08	0.05	2.02	48.57
11	82.15	*24.00	*0.07	1.82	50.18
12	82.14	15.22	0.05	0.77	49.29
13	80.86	*22.21	0.04	1.13	50.41
14	81.56	3.40	0.04	3.32	49.61
15	80.90	5.98	0.04	3.28	48.17
16	77.14	4.34	0.03	3.19	48.33
17	80.39	5.51	0.04	3.12	48.66
18	81.23	1.92	0.04	2.38	50.00
19	81.09	*28.65	0.05	2.02	51.25
20	79.44	0.01	0.00	2.86	*42.83
21	78.11	11.11	0.03	3.36	48.39
22	77.68	0.41	0.03	3.33	49.62
23	77.23	0.93	0.03	3.02	48.50

Nest #	ppm Chloride Jan 02	ppb Nitrite Jan 02	ppb Bromide Jan 02	ppb Nitrate Jan 02	ppm Sulfate Jan 02
24	76.77	10.00	0.03	3.09	47.84
25	*66.61	9.47	0.03	1.30	*32.92
26	81.54	3.11	0.04	3.53	49.62
27	83.52	3.85	0.04	3.55	50.15
28	77.28	0.78	0.03	2.94	48.90
29	*75.60	4.50	*0.18	0.71	*17.78
30	79.07	1.30	0.02	3.46	*52.07
31	79.78	8.87	0.04	3.13	50.26
32	81.68	1.31	0.04	3.57	49.42
33	80.32	0.44	0.04	3.54	48.73
34	76.52	0.55	0.02	3.09	48.40
35	78.82	2.28	0.04	3.24	49.42
36	77.39	0.39	0.02	3.18	49.09
37	77.27	10.36	*0.07	*0.19	46.69
38	82.87	1.84	0.04	3.66	50.60
39	83.95	3.00	0.04	2.68	49.72
40	81.58	0.01	*0.00	3.58	49.03
41	80.73	0.66	0.03	2.94	49.92
42	81.68	3.26	0.04	2.99	48.69
43	81.89	6.06	0.05	1.92	51.14
44	84.60	2.07	0.04	3.77	49.15
45	83.36	1.98	0.04	3.77	50.21
46	81.17	0.12	0.04	3.43	49.10
47	81.48	5.89	0.05	2.74	49.82
48	78.85	1.65	0.04	3.11	48.00
49	79.03	2.95	0.05	1.11	49.08
50	81.02	4.86	0.04	3.64	49.78
51	82.95	7.71	0.04	3.72	51.28
52	83.68	3.25	0.02	3.56	49.39
53	85.60	15.94	0.04	3.79	51.39
54	83.61	7.33	0.04	3.48	50.18
55	80.98	3.15	0.03	2.67	48.81
56	81.02	7.13	0.04	3.33	48.02
57	82.55	5.55	0.04	3.45	49.52
58	81.42	4.84	0.04	3.23	48.76
59	83.39	3.24	0.04	3.38	49.28
60	83.94	0.01	*0.00	3.46	49.85
61	80.28	3.06	0.05	3.04	47.84
62	82.12	4.62	0.04	3.37	49.70
63	80.09	2.98	0.04	2.87	47.48
64	81.27	3.29	0.04	3.25	48.54
65	83.06	4.87	0.03	3.36	49.36
66	81.55	2.14	0.02	3.08	48.27

Appendix AU: Data Results: Strata A, December 2002 (1st pass)

Nest #	ppb Fluoride Dec 02 (1)	ppb Lactate Dec 02 (1)	ppb Acetate Dec 02 (1)	ppb Propionate Dec 02 (1)	ppb Formate Dec 02 (1)	ppb Butyrate Dec 02 (1)
1	*483.36	207.33	0.00	0.00	*82.97	0.00
2	258.21	0.00	0.00	0.00	0.00	0.00
3	135.67	0.00	0.00	0.00	0.00	0.00
4	220.48	0.00	0.00	0.00	0.00	0.00
5	199.59	0.00	0.00	0.00	*34.45	0.00
6	190.50	0.00	0.00	*245.02	0.00	0.00
7	206.45	0.00	0.00	0.00	0.00	0.00
8	269.95	0.00	0.00	0.00	0.00	0.00
9	221.00	0.00	0.00	0.00	0.00	0.00
10	159.02	153.31	0.00	0.00	0.00	0.00
11	275.17	0.00	0.00	0.00	0.00	0.00
12	232.71	0.00	0.00	0.00	0.00	0.00
13	162.17	0.00	0.00	0.00	0.00	0.00
14	207.43	485.60	0.00	0.00	0.00	0.00
15	192.85	242.13	0.00	0.00	0.00	0.00
16	196.37	0.00	0.00	0.00	0.00	0.00
17	174.59	0.00	0.00	0.00	0.00	0.00
18	*896.65	0.00	0.00	0.00	0.00	0.00
19	174.17	199.50	0.00	*189.33	*54.11	0.00
20	228.25	234.61	0.00	0.00	*58.62	0.00
21	205.17	0.00	0.00	0.00	0.00	0.00
22	138.34	0.00	0.00	0.00	0.00	0.00
23	223.12	0.00	0.00	0.00	0.00	0.00
24	184.76	305.45	0.00	0.00	0.00	0.00
25	212.91	*518.86	0.00	0.00	*236.93	0.00
26	199.33	387.05	0.00	0.00	*83.09	0.00
27	266.70	*566.52	0.00	0.00	*80.88	0.00
28	174.26	445.30	0.00	0.00	0.00	0.00
29	185.76	0.00	0.00	0.00	0.00	0.00
30	153.95	0.00	0.00	0.00	0.00	0.00
31	179.22	299.60	0.00	0.00	0.00	0.00
32	160.44	0.00	0.00	0.00	0.00	0.00
33	175.75	0.00	0.00	0.00	0.00	0.00
34	193.71	0.00	0.00	0.00	*29.11	0.00
35	235.47	0.00	0.00	0.00	0.00	0.00
36	216.27	0.00	0.00	0.00	0.00	0.00
37	236.52	*2229.50	0.00	0.00	0.00	0.00
38	279.95	384.80	0.00	0.00	*91.13	0.00
39	242.38	239.18	0.00	0.00	*77.52	0.00
40	202.49	0.00	0.00	0.00	0.00	0.00
41	247.24	0.00	0.00	0.00	0.00	0.00
42	248.05	0.00	0.00	0.00	0.00	0.00
43	186.55	0.00	0.00	0.00	0.00	0.00
44	224.66	0.00	0.00	0.00	0.00	0.00

Nest #	ppb Fluoride Dec 02 (1)	ppb Lactate Dec 02 (1)	ppb Acetate Dec 02 (1)	ppb Propionate Dec 02 (1)	ppb Formate Dec 02 (1)	ppb Butyrate Dec 02 (1)
45	218.65	0.00	0.00	0.00	0.00	0.00
46	*317.03	0.00	0.00	0.00	0.00	0.00
47	238.02	0.00	0.00	0.00	0.00	0.00
48	185.44	0.00	0.00	0.00	0.00	0.00
49	219.29	0.00	0.00	0.00	0.00	0.00
50	181.34	0.00	0.00	0.00	0.00	0.00
51	179.14	0.00	0.00	0.00	0.00	0.00
52	209.96	0.00	0.00	0.00	0.00	0.00
53	172.64	0.00	0.00	0.00	0.00	0.00
54	217.27	0.00	0.00	0.00	0.00	0.00
55	179.08	136.44	0.00	0.00	0.00	0.00
56	*73.69	0.00	0.00	0.00	0.00	0.00
57	196.43	*545.69	0.00	0.00	*40.04	0.00
58	210.29	0.00	0.00	0.00	*76.97	0.00
59	175.98	0.00	0.00	0.00	0.00	0.00
60	142.63	0.00	0.00	0.00	0.00	0.00
61	187.51	202.34	0.00	0.00	*54.40	0.00
62	147.96	0.00	0.00	0.00	*58.91	0.00
63	207.87	*1105.22	0.00	0.00	0.00	0.00
64	194.43	0.00	0.00	0.00	0.00	0.00
65	141.63	*694.35	0.00	0.00	0.00	0.00
66	185.10	0.00	0.00	0.00	0.00	0.00

Nest #	ppm Chloride Dec 02 (1)	ppb Nitrite Dec 02 (1)	ppb Bromide Dec 02 (1)	ppm Nitrate Dec 02 (1)	ppm Sulfate Dec 02 (1)
1	*99.54	12.20	838.84	0.33	*15.13
2	72.20		0.00	*2.30	1.95
3	63.00		0.00	*2.56	3.04
4	74.95		0.00	0.55	0.05
5	74.08	0.00	2013.38	0.00	0.08
6	*125.89	0.00	80.59	0.03	0.36
7	87.59	0.00	0.00	*1.11	3.00
8	62.47	0.00	0.00	*3.08	0.19
9	67.03	0.00	146.54	0.25	*29.89
10	86.87	0.00	0.00	0.00	*27.01
11	68.65	0.00	69.69	*2.36	0.64
12	53.91	0.00	3706.69	0.15	0.15
13	65.23	0.00	2948.22	0.02	4.09
14	69.87	0.00	*3756.91	0.06	0.20
15	68.49	0.00	1332.54	0.03	1.12
16	65.50	0.00	32.98	0.02	*21.29
17	*44.76	0.00	0.00	*3.82	0.11
18	61.85	*95.70	*3724.41	0.00	0.35
19	64.42	18.40	60.93	0.05	*32.24
20	67.78	13.60	458.28	0.04	12.13
21	68.32	*56.80	46.54	0.39	*32.98
22	63.36	0.00	0.00	*2.44	*41.12
23	69.72	0.00	31.88	0.02	6.91

Nest #	ppm Chloride Dec 02 (1)	ppb Nitrite Dec 02 (1)	ppb Bromide Dec 02 (1)	ppm Nitrate Dec 02 (1)	ppm Sulfate Dec 02 (1)
24	70.41	*27.40	698.55	0.27	*23.14
25	58.78	9.90	1186.08	0.10	3.62
26	*158.79	0.00	3952.76	0.04	0.26
27	54.46	0.00	673.74	0.09	0.86
28	74.15	0.00	332.18	0.03	1.92
29	69.34	0.00	27.47	0.00	*29.51
30	64.96	0.00	0.00	0.00	*34.00
31	73.99	0.00	886.01	0.07	3.57
32	57.71	0.00	1678.37	0.10	0.26
33	65.57	0.00	128.14	0.07	3.19
34	70.33	0.00	838.84	0.33	6.02
35	66.42	0.00	0.00	*2.30	*30.63
36	66.66	24.40	0.00	*2.56	5.00
37	64.66	0.00	0.00	0.55	*22.36
38	51.14	0.00	2013.38	0.07	0.25
39	57.56	0.00	80.59	0.03	0.06
40	*128.80	0.00	0.00	*1.11	0.08
41	86.66	23.60	0.00	*3.08	0.95
42	71.68	0.00	146.54	0.26	3.31
43	86.15	0.00	0.00	0.00	2.56
44	67.59	*52.60	69.69	*2.36	2.70
45	*107.91	0.00	*3706.69	0.15	2.62
46	73.01	0.00	2948.22	0.02	0.08
47	93.02	0.00	3756.91	0.06	0.16
48	*138.64	0.00	1332.54	0.03	1.36
49	60.49	0.00	32.98	0.02	0.49
50	*223.91	0.00	0.00	*3.82	5.38
51	74.42	0.00	*3724.41	0.00	0.10
52	73.39	19.20	60.93	0.05	0.08
53	67.45	0.00	458.28	0.04	2.01
54	53.99	0.00	46.54	0.39	0.14
55	75.52	0.00	0.00	*2.44	6.28
56	*327.07	0.00	31.88	0.02	1.88
57	77.00	0.00	698.55	0.27	0.00
58	75.28	*27.40	1186.08	0.10	0.00
59	54.25	0.00	3952.76	0.04	0.00
60	59.00	0.00	673.74	0.09	0.47
61	63.25	0.00	332.18	0.03	3.86
62	*115.19	0.00	27.47	0.00	0.14
63	47.05	24.60	0.00	0.00	0.17
64	72.09	0.00	886.01	0.07	0.81
65	68.54	11.30	1678.37	0.10	0.51
66	73.91	0.00	128.14	0.07	1.06

Appendix AV: Data Results: Strata B, Dec 02 (1st pass)

Nest #	ppb Fluoride Dec 02 (1)	ppb Lactate Dec 02 (1)	ppb Acetate Dec 02 (1)	ppb Propionate Dec 02 (1)	ppb Formate Dec 02 (1)	ppb Butyrate Dec 02 (1)
1	244.74	493.52	0.00	0.00	122.46	0.00
2	321.06	323.34	0.00	0.00	138.23	0.00
3	338.24	638.60	0.00	0.00	33.23	0.00
4	249.11	0.00	0.00	0.00	26.17	0.00
5	485.62	*1043.29	0.00	*77.42	111.71	*89.81
6	344.06	0.00	0.00	0.00	102.96	0.00
7	234.73	337.24	0.00	*348.93	0.00	0.00
8	352.92	217.23	0.00	0.00	83.97	0.00
9	303.97	0.00	0.00	0.00	0.00	0.00
10	170.12	*1509.67	0.00	0.00	0.00	0.00
11	400.59	215.88	0.00	0.00	119.87	0.00
12	254.78	0.00	0.00	0.00	79.90	0.00
13	370.95	202.70	0.00	0.00	114.39	*45.14
14	362.72	647.32	0.00	0.00	71.29	*62.98
15	349.28	188.50	0.00	*105.43	83.40	0.00
16	276.42	677.38	0.00	*74.17	57.15	0.00
17	303.38	674.36	0.00	*123.34	34.34	0.00
18	444.47	193.92	0.00	0.00	103.23	0.00
19	194.88	453.79	0.00	0.00	108.67	0.00
20	255.80	261.48	0.00	0.00	54.86	0.00
21	143.52	0.00	0.00	*101.59	0.00	0.00
22	0.00	174.06	0.00	*38.86	68.00	0.00
23	0.00	0.00	0.00	0.00	0.00	0.00
24	197.81	696.49	0.00	0.00	105.94	0.00
25	196.00	775.86	0.00	*547.22	*289.32	0.00
26	192.49	0.00	0.00	0.00	72.20	0.00
27	195.63	0.00	0.00	0.00	*238.06	0.00
28	246.00	546.14	0.00	0.00	44.12	0.00
29	0.00	0.00	0.00	0.00	0.00	0.00
30	264.20	281.49	0.00	0.00	57.89	0.00
31	255.50	496.80	0.00	0.00	69.12	0.00
32	276.06	219.35	0.00	0.00	77.52	0.00
33	289.81	699.59	0.00	*84.50	47.92	0.00
34	0.00	0.00	0.00	0.00	0.00	0.00
35	243.35	0.00	0.00	0.00	0.00	0.00
36	203.32	0.00	0.00	*27.22	30.11	0.00
37	320.01	205.28	0.00	0.00	69.55	0.00
38	338.92	456.70	0.00	0.00	89.98	0.00
39	203.31	0.00	0.00	0.00	67.71	0.00
40	353.81	213.64	0.00	0.00	95.17	0.00
41	335.62	0.00	0.00	0.00	28.27	*43.53
42	373.27	0.00	0.00	0.00	52.29	0.00
43	0.00	240.07	0.00	0.00	48.51	0.00
44	0.00	0.00	0.00	0.00	42.12	*33.93

Nest #	ppb Fluoride Dec 02 (1)	ppb Lactate Dec 02 (1)	ppb Acetate Dec 02 (1)	ppb Propionate Dec 02 (1)	ppb Formate Dec 02 (1)	ppb Butyrate Dec 02 (1)
45	206.08	0.00	0.00	0.00	24.23	0.00
46	339.40	121.13	0.00	0.00	65.00	*57.99
47	262.97	124.84	0.00	*19.15	38.19	0.00
48	231.21	0.00	0.00	0.00	0.00	0.00
49	249.43	0.00	0.00	0.00	63.83	0.00
50	241.34	0.00	0.00	0.00	0.00	*76.96
51	201.57	0.00	0.00	0.00	42.41	0.00
52	0.00	124.00	0.00	0.00	65.88	0.00
53	0.00	133.19	0.00	0.00	50.12	0.00
54	0.00	140.48	0.00	0.00	31.42	0.00
55	367.90	360.69	0.00	0.00	101.87	0.00
56	141.01	0.00	0.00	0.00	0.00	0.00
57	258.19	0.00	0.00	0.00	53.72	0.00
58	244.32	313.77	0.00	0.00	25.85	0.00
59	314.20	122.05	0.00	0.00	53.90	*63.31
60	294.63	157.55	0.00	0.00	85.00	0.00
61	0.00	136.49	0.00	0.00	78.31	0.00
62	221.15	164.15	0.00	0.00	52.54	0.00
63	178.21	340.44	0.00	*172.57	93.75	0.00
64	381.95	139.07	0.00	0.00	43.50	0.00
65	192.71	576.18	0.00	0.00	0.00	0.00
66	279.41	405.82	0.00	0.00	60.51	0.00

Nest #	ppm Chloride Dec 02 (1)	ppb Nitrite Dec 02 (1)	ppb Bromide Dec 02 (1)	ppm Nitrate Dec 02 (1)	ppm Sulfate Dec 02 (1)
1	61.44	35.99	2006.78	0.07	0.41
2	57.23	0.00	1695.15	0.07	*85.19
3	64.65	0.00	316.80	0.02	6.53
4	66.92	0.00	91.02	0.03	20.60
5	59.75	0.00	799.61	0.02	0.17
6	53.49	*47.45	1600.38	0.12	10.60
7	64.17	16.06	168.33	0.00	20.20
8	64.85	0.00	1758.45	0.00	0.11
9	63.88	0.00	67.11	0.02	34.36
10	67.18		0.00		*42.51
11	62.02	30.17	1679.70	0.02	0.25
12	51.23	39.48	1415.92	0.02	0.26
13	60.71	0.00	1852.16	0.00	0.15
14	60.14	0.00	1085.39	0.00	0.65
15	61.75	0.00	3370.95	0.05	0.33
16	67.51	0.00	850.73	0.00	1.14
17	44.03	*129.34	3603.32	0.22	1.07
18	57.22	0.00	1925.01	0.00	0.13
19	60.00	*52.55	1141.43	0.06	1.46
20	59.69	16.50	1440.66	0.02	2.26
21	62.47	*120.48	50.57	*0.51	*30.09
22	65.33	*191.36	34.40	*0.54	*32.92
23	64.34	*128.89	43.63	0.20	15.46

Nest #	ppm Chloride Dec 02 (1)	ppb Nitrite Dec 02 (1)	ppb Bromide Dec 02 (1)	ppm Nitrate Dec 02 (1)	ppm Sulfate Dec 02 (1)
24	51.54	*86.22	2468.79	0.19	1.51
25	61.03	0.00	497.10	*0.28	23.50
26	49.11	0.00	2432.77	0.05	1.07
27	46.06	0.00	3058.94	0.00	0.14
28	65.43	*55.04	185.43	0.06	1.40
29	64.18	0.00	53.24	0.05	25.76
30	59.08	0.00	326.46	0.20	21.47
31	78.54	0.00	1603.11	*0.25	20.69
32	61.58	*43.53	1067.73	0.13	1.11
33	66.38	23.31	296.64	0.00	0.93
34	41.33		136.18	*4.50	13.51
35	63.94	0.00	0.00	0.03	*34.91
36	64.95	0.00	48.32	*0.41	9.40
37	63.82	0.00	368.82	0.05	20.10
38	52.86	0.00	2950.54	0.00	0.13
39	63.35	19.73	1071.27	0.03	7.42
40	63.09	0.00	2249.44	0.05	0.07
41	65.67	0.00	284.00	0.00	9.81
42	69.86	0.00	353.05	*0.64	0.14
43	56.36	0.00	3374.46	0.05	0.88
44	53.58	0.00	3287.01	0.07	0.19
45	64.10	0.00	477.76	0.02	26.03
46	50.44	0.00	3514.38	0.09	1.82
47	44.21	17.97	2897.63	0.10	3.38
48	41.73	0.00	2797.35	0.00	0.16
49	*91.02	10.62	3823.77	0.00	2.07
50	63.54	0.00	4103.91	0.09	0.22
51	67.67	0.00	447.86	0.00	2.47
52	66.95	0.00	972.87	0.05	2.04
53	63.57	14.61	853.28	0.04	0.16
54	61.54	0.00	2573.07	0.09	0.20
55	55.25	0.00	4292.10	0.02	0.42
56	*37.10	0.00	5095.18	0.00	0.59
57	68.39	0.00	897.50	0.11	0.13
58	66.75	0.00	450.75	0.00	2.59
59	42.56	0.00	4023.72	0.08	0.18
60	65.97	0.00	1100.23	0.04	11.08
61	45.06	0.00	3319.43	0.05	4.35
62	60.67	0.00	1383.77	0.00	6.82
63	45.65	0.00	2935.69	0.00	0.10
64	63.85	22.05	551.16	0.00	9.23
65	64.45	0.00	71.23	0.01	0.19
66	70.52	0.00	217.59	0.00	0.87

Appendix AW: Data Results: Strata C, Dec 02 (1st pass)

Nest #	ppb Fluoride Dec 02 (1)	ppb Lactate Dec 02 (1)	ppb Acetate Dec 02 (1)	ppb Propionate Dec 02 (1)	ppb Formate Dec 02 (1)	ppb Butyrate Dec 02 (1)
1	156.96	0.00	0.00	0.00	0.00	0.00
2	143.13	0.00	0.00	0.00	0.00	0.00
3	159.59	0.00	0.00	0.00	0.00	0.00
4	148.46	0.00	0.00	0.00	0.00	0.00
5	159.35	0.00	0.00	0.00	0.00	0.00
6						
7	178.26	*67.74	0.00	0.00	*31.73	0.00
8	167.40	0.00	0.00	0.00	0.00	0.00
9	180.18	0.00	0.00	0.00	0.00	0.00
10	159.50	0.00	0.00	0.00	0.00	0.00
11	171.05	*22.42	0.00	0.00	0.00	0.00
12	147.19	0.00	0.00	0.00	0.00	0.00
13	142.41	0.00	0.00	0.00	0.00	0.00
14	151.46	0.00	0.00	0.00	0.00	0.00
15	*0.00	0.00	0.00	0.00	0.00	0.00
16	134.20	0.00	0.00	0.00	0.00	0.00
17	146.68	0.00	0.00	0.00	0.00	0.00
18	170.49	0.00	0.00	0.00	0.00	0.00
19	178.10	0.00	0.00	0.00	0.00	0.00
20	169.63	0.00	0.00	0.00	0.00	0.00
21	*0.00	0.00	0.00	0.00	0.00	0.00
22	110.22	0.00	0.00	0.00	0.00	0.00
23	167.12	0.00	0.00	0.00	0.00	0.00
24	170.91	0.00	0.00	0.00	0.00	0.00
25	158.82	0.00	0.00	0.00	0.00	0.00
26	164.32	0.00	0.00	0.00	0.00	0.00
27	166.49	0.00	0.00	0.00	0.00	0.00
28	180.22	0.00	0.00	0.00	0.00	0.00
29	171.40	0.00	0.00	0.00	*27.56	0.00
30	163.14	0.00	0.00	0.00	0.00	0.00
31	193.09	0.00	0.00	0.00	0.00	0.00
32	161.09	0.00	0.00	0.00	0.00	0.00
33	172.05	0.00	0.00	0.00	0.00	0.00
34	146.25	0.00	0.00	0.00	0.00	0.00
35	167.38	0.00	0.00	0.00	0.00	0.00
36	162.96	0.00	0.00	0.00	0.00	0.00
37	149.61	0.00	0.00	0.00	0.00	0.00
38	164.55	0.00	0.00	0.00	0.00	0.00
39	166.49	0.00	0.00	0.00	0.00	0.00
40	154.67	0.00	0.00	0.00	0.00	0.00
41	166.76	0.00	0.00	0.00	0.00	0.00
42	158.66	0.00	0.00	0.00	0.00	0.00
43	161.47	0.00	0.00	0.00	0.00	0.00
44	162.44	0.00	0.00	0.00	0.00	0.00

Nest #	ppb Fluoride Dec 02 (1)	ppb Lactate Dec 02 (1)	ppb Acetate Dec 02 (1)	ppb Propionate Dec 02 (1)	ppb Formate Dec 02 (1)	ppb Butyrate Dec 02 (1)
45	*196.51	0.00	0.00	0.00	0.00	0.00
46	*243.63	0.00	0.00	0.00	0.00	0.00
47	164.45	0.00	0.00	0.00	0.00	0.00
48	161.19	0.00	0.00	0.00	0.00	0.00
49	164.13	0.00	0.00	0.00	0.00	0.00
50	176.79	0.00	0.00	0.00	0.00	0.00
51	161.12	0.00	0.00	0.00	0.00	0.00
52	153.90	0.00	0.00	0.00	0.00	0.00
53	185.00	0.00	0.00	0.00	0.00	0.00
54	171.39	0.00	0.00	0.00	0.00	0.00
55	168.09	0.00	0.00	0.00	0.00	0.00
56	169.21	0.00	0.00	0.00	0.00	0.00
57	167.79	0.00	0.00	0.00	0.00	0.00
58	153.54	0.00	0.00	0.00	0.00	0.00
59	170.12	0.00	0.00	0.00	0.00	0.00
60	*198.23	0.00	0.00	0.00	0.00	0.00
61	154.49	0.00	0.00	0.00	0.00	0.00
62	143.48	0.00	0.00	0.00	0.00	0.00
63	163.36	0.00	0.00	0.00	0.00	0.00
64	170.50	0.00	0.00	0.00	0.00	0.00
65	176.46	0.00	0.00	0.00	0.00	0.00
66	181.48	0.00	0.00	0.00	0.00	0.00

Nest #	ppm Chloride Dec 02 (1)	ppb Nitrite Dec 02 (1)	ppb Bromide Dec 02 (1)	ppm Nitrate Dec 02 (1)	ppm Sulfate Dec 02 (1)
1	64.16	16.72	8.59	9.10	36.72
2	65.45	45.18	21.39	8.39	37.12
3	67.68	98.07	21.47	4.52	37.18
4	66.82	44.69	0.00	6.46	38.38
5	63.14	41.41	13.50	7.29	36.23
6					
7	65.49	139.57	21.00	6.50	37.34
8	65.33	128.55	20.59	6.66	37.63
9	66.77	27.48	19.07	8.47	38.10
10	63.04	27.25	19.50	9.16	35.97
11	64.02	124.68	33.16	7.60	35.41
12	63.12	27.94	11.52	6.31	35.66
13	62.27	40.34	23.62	6.06	36.64
14	60.43	20.92	0.00	9.72	*34.21
15	63.13	19.20	0.00	5.43	35.96
16	62.96	36.27	0.00	12.02	36.03
17	60.18	210.23	0.00	6.48	35.69
18	63.11	31.28	0.00	10.50	35.64
19	71.58	0.00	18.80	7.81	39.57
20	68.34	*434.89	15.43	11.41	37.11
21	69.27	*837.44	22.34	11.57	38.22
22	66.68	0.00	16.71	12.00	36.35
23	69.80	0.00	18.28	12.71	39.52

Nest #	ppm Chloride Dec 02 (1)	ppb Nitrite Dec 02 (1)	ppb Bromide Dec 02 (1)	ppm Nitrate Dec 02 (1)	ppm Sulfate Dec 02 (1)
24	76.56	*503.51	23.56	7.80	*42.98
25	69.26	61.86	19.25	8.30	39.71
26	73.38	367.94	24.71	9.83	38.44
27	75.43	224.12	0.00	7.24	39.10
28	76.26	10.67	0.00	9.95	*45.24
29	*86.09	0.00	0.00	0.00	*4.75
30	71.19	160.57	17.23	9.32	37.77
31	72.33	227.61	14.70	8.75	39.71
32	66.95	116.00	0.00	11.77	37.64
33	67.59	*519.72	23.22	6.28	37.33
34	66.43	0.00	0.00	13.04	38.75
35	70.46	132.87	24.16	8.40	39.41
36	*85.13	36.09	26.39	13.06	39.50
37	79.27	3.49	*81.38	2.22	40.83
38	78.55	0.00	*50.85	11.50	38.18
39	76.36	404.59	9.20	9.14	38.36
40	72.10	0.00	21.62	3.08	39.36
41	72.56	294.49	23.74	8.14	40.49
42	70.52	261.11	20.43	11.56	38.60
43	69.83	269.05	0.00	5.35	38.84
44	69.48	201.14	20.45	12.04	37.40
45	68.67	231.80	23.23	11.03	*42.55
46	71.88	*492.16	20.19	9.51	40.48
47	73.15	0.00	10.32	12.31	37.18
48	73.78	0.00	0.00	12.19	38.74
49	70.78	0.00	39.94	0.44	38.42
50	68.60	0.00	21.92	12.20	37.96
51	67.74	0.00	19.03	12.14	37.55
52	69.14	11.76	17.81	10.93	38.27
53	69.85	47.82	21.54	12.79	39.05
54	67.80	111.51	20.13	10.50	38.14
55	68.49	69.62	23.06	1.57	37.68
56	68.75	0.00	0.00	11.87	38.16
57	67.96	52.77	21.90	10.56	37.90
58	65.78	163.52	22.15	7.13	37.03
59	66.43	0.00	14.12	10.96	37.08
60	68.79	0.00	8.19	9.86	38.45
61	68.66	60.00	27.67	10.07	37.83
62	68.77	*436.94	22.70	10.21	38.29
63	68.97	132.64	28.88	1.31	38.44
64	70.14	0.00	11.84	9.98	38.81
65	69.61	38.74	28.00	8.06	38.49
66	68.87	24.27	15.13	12.18	38.49

Appendix AX: Data Results: Strata A, Dec 02 (2nd pass)

Nest #	ppb Fluoride Dec 02 (2)	ppb Lactate Dec 02 (2)	ppb Acetate Dec 02 (2)	ppb Propionate Dec 02 (2)	ppb Formate Dec 02 (2)	ppb Butyrate Dec 02 (2)
1	210.73	0.00	0.00	0.00	0.00	0.00
2	202.74	0.00	0.00	0.00	54.25	0.00
3	190.07	0.00	0.00	0.00	70.99	0.00
4	186.64	0.00	0.00	0.00	42.63	0.00
5	*11.45	0.00	0.00	0.00	47.97	0.00
6	218.54	0.00	0.00	0.00	52.67	0.00
7	203.04	0.00	0.00	0.00	48.53	0.00
8	208.66	0.00	0.00	0.00	0.00	0.00
9	292.79	0.00	0.00	0.00	37.75	0.00
10	356.31	0.00	0.00	0.00	21.99	0.00
11	154.77	0.00	0.00	0.00	66.53	0.00
12	287.82	0.00	0.00	0.00	0.00	0.00
13	193.19	0.00	0.00	0.00	65.41	0.00
14	*11.80	*89.82	0.00	0.00	32.75	0.00
15	302.55	0.00	0.00	0.00	27.35	0.00
16	266.03	0.00	0.00	0.00	43.38	0.00
17	242.84	0.00	0.00	0.00	0.00	0.00
18	156.41	0.00	0.00	0.00	0.00	0.00
19	249.99	0.00	0.00	0.00	56.97	0.00
20	180.54	0.00	0.00	0.00	0.00	0.00
21	231.05	0.00	0.00	0.00	57.40	0.00
22	66.79	0.00	0.00	0.00	23.75	0.00
23	*7.58	0.00	0.00	0.00	0.00	0.00
24	219.46	0.00	0.00	0.00	45.77	0.00
25	*14.68	0.00	0.00	0.00	0.00	0.00
26	179.80	0.00	0.00	0.00	49.49	0.00
27	260.14	0.00	0.00	0.00	29.60	0.00
28	191.00	0.00	0.00	0.00	20.69	0.00
29	217.07	0.00	0.00	0.00	35.21	0.00
30	209.96	0.00	0.00	0.00	0.00	0.00
31	*18.66	0.00	0.00	0.00	0.00	0.00
32	250.75	0.00	0.00	0.00	31.06	0.00
33	268.50	0.00	0.00	0.00	29.42	0.00
34	303.00	0.00	0.00	0.00	0.00	0.00
35	289.45	0.00	0.00	0.00	0.00	0.00
36	*21.39	0.00	0.00	0.00	0.00	0.00
37	268.37	0.00	0.00	0.00	0.00	0.00
38	256.10	0.00	0.00	0.00	44.45	0.00
39	270.27	0.00	0.00	0.00	29.19	0.00
40	188.67	0.00	0.00	0.00	29.47	0.00
41	223.35	0.00	0.00	0.00	0.00	0.00
42	231.39	0.00	0.00	0.00	0.00	0.00
43	246.19	0.00	0.00	0.00	0.00	0.00
44	296.70	0.00	0.00	0.00	0.00	0.00

Nest #	ppb Fluoride Dec 02 (2)	ppb Lactate Dec 02 (2)	ppb Acetate Dec 02 (2)	ppb Propionate Dec 02 (2)	ppb Formate Dec 02 (2)	ppb Butyrate Dec 02 (2)
45	131.24	0.00	0.00	0.00	0.00	0.00
46	252.25	0.00	0.00	0.00	0.00	0.00
47	206.62	0.00	0.00	0.00	0.00	0.00
48	*18.88	0.00	0.00	0.00	0.00	0.00
49	255.54	0.00	0.00	0.00	0.00	0.00
50	234.96	0.00	0.00	0.00	17.92	0.00
51	332.88	0.00	0.00	0.00	0.00	0.00
52	249.47	0.00	0.00	0.00	0.00	0.00
53	278.65	0.00	0.00	0.00	0.00	0.00
54	208.68	*17.75	0.00	0.00	20.38	0.00
55	296.65	0.00	0.00	0.00	0.00	0.00
56	235.95	0.00	0.00	0.00	16.85	0.00
57	282.17	0.00	0.00	0.00	0.00	0.00
58	306.51	*1255.98	0.00	0.00	0.00	0.00
59	267.65	0.00	0.00	0.00	29.06	0.00
60	249.38	0.00	0.00	0.00	27.36	0.00
61	*10.45	0.00	0.00	0.00	13.86	0.00
62	163.55	0.00	0.00	0.00	0.00	0.00
63	198.91	0.00	0.00	0.00	10.96	0.00
64	276.74	0.00	0.00	0.00	0.00	0.00
65	*13.91	0.00	0.00	0.00	0.00	0.00
66	225.71	0.00	0.00	0.00	0.00	0.00

Nest #	ppm Chloride Dec 02 (2)	ppb Nitrite Dec 02 (2)	ppb Bromide Dec 02 (2)	ppm Nitrate Dec 02 (2)	ppm Sulfate Dec 02 (2)
1	80.30	0.00	52.38	*3.24	*38.69
2	66.70	0.00	23.38	0.54	33.01
3	74.14	0.00	25.62	*2.76	35.57
4	74.15	0.00	34.50	*1.61	*38.33
5	*7.20	0.00	0.00	0.21	3.72
6	77.26	0.00	49.67	*1.00	*39.60
7	72.07	0.00	26.72	*3.69	35.46
8	76.11	0.00	0.00	*3.65	*53.11
9	83.34	18.24	119.40	0.10	1.71
10	82.52	0.00	119.76	0.09	15.55
11	65.06	76.92	502.19	*1.81	4.16
12	*156.46	0.00	*1928.58	*2.33	8.39
13	79.17	0.00	146.21	*2.00	12.29
14	*5.36	0.00	0.00	0.10	1.72
15	*119.72	0.00	190.17	0.12	6.34
16	68.51	0.00	*1950.99	0.14	0.34
17	71.47	42.77	1203.67	0.15	4.04
18	60.22	0.00	29.18	0.43	31.98
19	68.74	0.00	116.01	*2.29	13.40
20	73.16	0.00	71.51	0.32	25.76
21	78.55	35.87	165.48	*1.08	5.10
22	38.47	0.00	1011.42	*1.00	0.90
23	*4.16	0.00	49.73	0.03	0.31

Nest #	ppm Chloride Dec 02 (2)	ppb Nitrite Dec 02 (2)	ppb Bromide Dec 02 (2)	ppm Nitrate Dec 02 (2)	ppm Sulfate Dec 02 (2)
24	77.02	0.00	43.71	0.39	34.73
25	*5.64	0.00	0.00	0.02	0.42
26	70.87	0.00	46.91	0.36	28.79
27	99.94	0.00	260.59	0.18	0.36
28	58.14	0.00	761.21	0.06	0.73
29	74.43	0.00	264.50	0.12	2.44
30	76.73	0.00	44.57	0.73	*47.83
31	*7.70	112.36	0.00	0.10	2.21
32	79.23	0.00	108.81	0.23	7.54
33	70.19	217.86	224.40	0.45	5.72
34	95.36	0.00	1435.18	0.14	0.22
35	84.66	15.61	958.67	0.07	0.37
36	*10.35	0.00	0.00	0.03	4.62
37	83.36	0.00	109.72	0.20	14.89
38	78.18	0.00	542.72	0.09	4.77
39	*133.00	0.00	782.99	0.12	4.73
40	51.58	0.00	1100.55	0.11	0.82
41	86.61	0.00	*1668.58	0.09	0.40
42	83.94	12.50	164.90	0.09	19.53
43	89.89	0.00	139.68	0.14	4.97
44	104.45	0.00	506.08	0.12	0.31
45	49.02	*5193.57	858.86	*1.41	2.80
46	113.24	0.00	1347.50	0.49	1.24
47	76.17	10.81	638.37	0.11	0.25
48	12.97	0.00	0.00	0.17	6.21
49	77.61	0.00	23.61	0.64	22.88
50	65.92	0.00	577.24	0.06	0.20
51	93.03	0.00	1353.66	0.14	0.36
52	53.31	0.00	*2389.16	0.12	0.39
53	110.34	0.00	924.64	0.22	0.17
54	73.86	0.00	0.00	0.19	35.01
55	80.15	0.00	233.75	0.28	8.53
56	74.02	0.00	400.90	*14.29	12.47
57	85.30	42.48	351.67	0.46	2.45
58	236.30	0.00	1473.43	0.09	0.07
59	85.93	20.96	394.20	0.17	0.32
60	82.28	0.00	116.91	0.52	8.94
61	5.04	0.00	0.00	0.07	2.17
62	54.77	10.33	65.45	0.07	1.54
63	61.80	15.98	229.47	0.17	0.24
64	77.65	0.00	0.00	0.11	1.01
65	6.39	0.00	0.00	0.00	0.29
66	68.10	0.00	57.59	0.29	13.44

Appendix AY: Data Results: Strata B, Dec 02 (2nd pass)

Nest #	ppb Fluoride Dec 02 (2)	ppb Lactate Dec 02 (2)	ppb Acetate Dec 02 (2)	ppb Propionate Dec 02 (2)	ppb Formate Dec 02 (2)	ppb Butyrate Dec 02 (2)
1	262.98	437.33	0.00	0.00	135.17	0.00
2	*0.00	0.00	0.00	0.00	0.00	0.00
3	216.20	299.91	0.00	*53.71	140.03	0.00
4	283.16	0.00	0.00	*211.21	0.00	0.00
5	279.28	129.48	0.00	0.00	171.08	0.00
6	264.64	117.34	0.00	0.00	155.40	0.00
7	213.05	211.17	0.00	0.00	91.53	0.00
8	285.08	341.51	0.00	0.00	75.92	0.00
9	226.39	92.80	0.00	0.00	98.42	0.00
10	222.51	0.00	0.00	0.00	65.35	0.00
11	173.92	0.00	0.00	0.00	0.00	0.00
12	245.17	175.96	0.00	0.00	150.01	0.00
13	278.44	221.06	0.00	0.00	128.38	0.00
14	184.49	*895.76	0.00	0.00	142.75	0.00
15	224.05	436.26	0.00	*71.32	112.24	0.00
16	209.76	*765.57	0.00	0.00	166.13	0.00
17	287.89	232.25	0.00	0.00	127.03	0.00
18	266.57	278.34	0.00	0.00	169.44	0.00
19	192.58	445.26	0.00	0.00	147.84	0.00
20	226.51	316.37	0.00	0.00	96.97	0.00
21	201.05	126.99	0.00	0.00	155.36	0.00
22	168.72	0.00	0.00	0.00	123.11	0.00
23	191.25	0.00	0.00	0.00	113.31	0.00
24	*26.56	38.98	0.00	0.00	0.00	0.00
25	213.05	*734.58	0.00	*86.46	113.65	0.00
26	248.64	96.46	0.00	0.00	159.71	0.00
27	244.04	267.69	0.00	0.00	194.43	0.00
28	172.65	*650.26	0.00	*128.81	100.31	0.00
29	187.38	0.00	0.00	0.00	73.97	0.00
30	174.62	203.85	0.00	0.00	89.35	0.00
31	159.08	532.13	0.00	0.00	92.77	0.00
32	190.53	213.32	0.00	0.00	49.68	0.00
33	157.52	122.93	0.00	0.00	126.60	0.00
34	211.18	*1808.27	0.00	*101.27	89.73	*118.12
35	176.52	0.00	0.00	0.00	69.55	0.00
36	227.76	198.97	0.00	0.00	72.86	0.00
37	177.52	67.57	0.00	0.00	111.53	0.00
38	181.64	0.00	0.00	*32.84	0.00	0.00
39	163.50	0.00	0.00	0.00	0.00	0.00
40	191.71	0.00	0.00	0.00	0.00	0.00
41	186.62	88.91	0.00	0.00	0.00	0.00
42	169.08	138.25	0.00	0.00	0.00	0.00
43	189.95	108.25	0.00	0.00	257.15	0.00
44	164.38	0.00	0.00	0.00	66.33	0.00

Nest #	ppb Fluoride Dec 02 (2)	ppb Lactate Dec 02 (2)	ppb Acetate Dec 02 (2)	ppb Propionate Dec 02 (2)	ppb Formate Dec 02 (2)	ppb Butyrate Dec 02 (2)
45	191.96	72.54	0.00	0.00	109.67	0.00
46	252.66	333.48	0.00	0.00	271.64	0.00
47	*24.52	0.00	0.00	0.00	62.84	0.00
48	199.97	0.00	0.00	0.00	0.00	0.00
49	168.08	0.00	0.00	0.00	0.00	0.00
50	159.71	0.00	0.00	0.00	0.00	0.00
51	246.45	141.55	0.00	0.00	122.97	0.00
52	258.01	212.75	0.00	0.00	238.39	0.00
53	242.91	0.00	0.00	0.00	215.43	0.00
54	205.58	0.00	0.00	0.00	163.95	0.00
55	*0.00	0.00	0.00	0.00	88.42	0.00
56	207.51	135.00	0.00	0.00	114.64	0.00
57	229.49	212.96	0.00	0.00	127.52	0.00
58	192.56	0.00	0.00	0.00	0.00	0.00
59	209.25	0.00	0.00	0.00	54.64	0.00
60	206.82	0.00	0.00	0.00	270.42	0.00
61	147.12	136.26	0.00	0.00	144.21	0.00
62	160.12	0.00	0.00	0.00	234.00	0.00
63	*5.21	0.00	0.00	0.00	0.00	0.00
64	169.94	238.01	0.00	0.00	106.46	0.00
65	*0.00	0.00	0.00	0.00	0.00	0.00
66	184.94	0.00	0.00	0.00	70.51	0.00

Nest #	ppm Chloride Dec 02 (2)	ppb Nitrite Dec 02 (2)	ppb Bromide Dec 02 (2)	ppm Nitrate Dec 02 (2)	ppm Sulfate Dec 02 (2)
1	56.03	0.00	2155.56	0.18	0.50
2	63.99	0.00	1673.40	0.00	*84.93
3	68.52	0.00	191.76	0.09	19.36
4	71.63	0.00	0.00	0.00	30.97
5	60.50	0.00	861.53	0.07	0.24
6	56.95	0.00	1991.72	0.11	4.35
7	74.18	0.00	145.50	0.05	19.87
8	69.84	0.00	1834.18	0.12	0.23
9	71.25	0.00	56.23	0.07	32.99
10	70.88	*128.93	269.19	0.00	44.15
11	61.30	0.00	786.26	0.07	0.67
12	54.80	*50.05	1363.91	0.10	0.21
13	74.63	*24.83	2021.10	0.00	0.23
14	67.83	0.00	710.91	0.06	5.12
15	67.20	0.00	3375.89	0.10	0.15
16	64.97	0.00	830.68	0.06	0.32
17	49.62	0.00	3847.76	0.10	0.33
18	63.78	0.00	2125.29	0.11	0.32
19	65.67	*15.21	898.47	0.05	12.22
20	62.39	0.00	1093.24	0.05	7.15
21	68.86	*121.44	95.96	*0.39	26.96
22	63.57	*30.74	27.21	0.10	22.77
23	68.88	0.00	35.23	0.04	29.17

Nest #	ppm Chloride Dec 02 (2)	ppb Nitrite Dec 02 (2)	ppb Bromide Dec 02 (2)	ppm Nitrate Dec 02 (2)	ppm Sulfate Dec 02 (2)
24	*7.92	0.00	336.14	0.02	0.22
25	68.66	0.00	384.99	0.05	23.10
26	49.75	0.00	2600.61	0.12	0.45
27	46.08	0.00	2726.74	0.12	0.24
28	70.74	0.00	152.70	0.08	0.91
29	72.15	0.00	39.03	0.10	26.38
30	66.99	0.00	273.90	0.12	22.49
31	55.76	0.00	1128.66	0.09	3.17
32	64.25	0.00	1015.08	0.08	0.50
33	63.61	0.00	205.25	0.08	4.74
34	69.60	0.00	298.11	0.04	0.34
35	67.80	0.00	0.00	0.07	33.33
36	71.49	0.00	0.00	0.06	18.17
37	65.88	0.00	363.43	0.07	18.90
38	58.45	*20.23	2986.47	0.00	0.22
39	65.80	0.00	1072.91	0.08	2.76
40	64.83	0.00	2045.01	0.00	0.24
41	58.20	0.00	296.39	0.04	2.52
42	50.47	0.00	260.78	0.06	0.13
43	61.50	0.00	2505.22	0.13	7.52
44	61.45	*29.06	1987.11	*0.31	9.34
45	64.09	0.00	357.03	0.04	24.35
46	56.93	0.00	3352.64	0.15	1.44
47	*10.16	0.00	497.33	*0.23	1.57
48	*28.27	0.00	2655.67	0.09	0.30
49	89.47	*86.38	3855.20	*0.22	0.95
50	65.24	0.00	3925.89	0.05	0.38
51	75.12	0.00	740.34	0.06	1.30
52	72.73	0.00	805.36	0.06	3.09
53	71.63	0.00	707.88	0.08	0.14
54	64.97	0.00	2370.72	0.11	0.24
55	*6.10	0.00	413.59	0.06	0.97
56	72.39	0.00	756.71	0.07	0.11
57	70.58	0.00	243.24	0.07	4.93
58	47.33	0.00	4159.56	0.08	0.16
59	70.87	0.00	1356.69	0.09	2.73
60	51.16	0.00	3804.32	0.07	2.11
61	71.53	0.00	138.64	0.06	27.24
62	58.73	0.00	2241.10	0.12	0.30
63	*2.62	0.00	0.00	0.05	0.99
64	74.14	0.00	67.90	0.00	1.96
65	94.31	0.00	145.22	0.00	0.83
66	68.08	0.00	0.00	12.49	38.75

Appendix AZ: Data Results: Strata C, December 02 (2nd pass)

Nest #	ppb Fluoride Dec 02 (2)	ppb Lactate Dec 02 (2)	ppb Acetate Dec 02 (2)	ppb Propionate Dec 02 (2)	ppb Formate Dec 02 (2)	ppb Butyrate Dec 02 (2)
1	170.97	0.00	0.00	0.00	43.52	0.00
2	179.85	0.00	0.00	0.00	64.34	0.00
3	177.59	0.00	0.00	0.00	16.95	0.00
4	175.64	0.00	0.00	0.00	79.50	0.00
5	204.43	0.00	0.00	0.00	107.58	0.00
6	unavailable	unavailable	unavailable	unavailable	unavailable	unavailable
7	176.90	0.00	0.00	0.00	0.00	0.00
8	184.16	0.00	0.00	0.00	47.38	0.00
9	180.84	0.00	0.00	0.00	51.12	0.00
10	171.16	0.00	0.00	0.00	49.10	0.00
11	179.06	0.00	0.00	0.00	81.31	0.00
12	160.91	0.00	0.00	0.00	32.05	0.00
13	171.57	0.00	0.00	0.00	105.71	0.00
14	*227.28	0.00	0.00	0.00	0.00	0.00
15	190.34	0.00	0.00	0.00	70.26	0.00
16	162.64	0.00	0.00	0.00	78.96	0.00
17	166.89	0.00	0.00	0.00	45.43	0.00
18	165.71	0.00	0.00	0.00	75.24	0.00
19	171.75	0.00	0.00	0.00	0.00	0.00
20	170.63	0.00	0.00	0.00	43.16	0.00
21	179.92	0.00	0.00	0.00	32.25	0.00
22	167.52	0.00	0.00	0.00	62.84	0.00
23	169.50	0.00	0.00	0.00	31.57	0.00
24	*35.96	0.00	0.00	0.00	0.00	0.00
25	165.62	0.00	0.00	0.00	31.10	0.00
26	173.62	0.00	0.00	0.00	75.12	0.00
27	201.31	0.00	0.00	0.00	58.17	0.00
28	185.93	0.00	0.00	0.00	40.27	0.00
29	215.04	0.00	0.00	0.00	78.11	0.00
30	188.90	0.00	0.00	0.00	45.37	0.00
31	205.83	0.00	0.00	0.00	32.29	0.00
32	154.47	0.00	0.00	0.00	37.87	0.00
33	170.31	0.00	0.00	0.00	46.79	0.00
34	*87.45	0.00	0.00	0.00	29.36	0.00
35	171.40	0.00	0.00	0.00	50.58	0.00
36	174.75	0.00	0.00	0.00	51.18	0.00
37	165.14	0.00	0.00	0.00	59.56	0.00
38	199.95	0.00	0.00	0.00	79.70	0.00
39	168.18	0.00	0.00	0.00	53.83	0.00
40	189.92	0.00	0.00	0.00	51.23	0.00
41	180.83	0.00	0.00	0.00	67.24	0.00
42	184.81	0.00	0.00	0.00	45.02	0.00
43	175.92	0.00	0.00	0.00	67.39	0.00
44	178.58	0.00	0.00	0.00	77.31	0.00

Nest #	ppb Fluoride Dec 02 (2)	ppb Lactate Dec 02 (2)	ppb Acetate Dec 02 (2)	ppb Propionate Dec 02 (2)	ppb Formate Dec 02 (2)	ppb Butyrate Dec 02 (2)
45	179.09	0.00	0.00	0.00	0.00	0.00
46	180.86	0.00	0.00	0.00	45.27	0.00
47	199.66	0.00	0.00	0.00	53.13	0.00
48	182.80	0.00	0.00	0.00	64.59	0.00
49	157.47	0.00	0.00	0.00	20.96	0.00
50	200.10	0.00	0.00	0.00	64.31	0.00
51	175.67	0.00	0.00	0.00	43.19	0.00
52	140.80	0.00	0.00	0.00	67.62	0.00
53	*229.76	0.00	0.00	0.00	44.05	0.00
54	164.73	0.00	0.00	0.00	31.74	0.00
55	173.69	*33.27	0.00	0.00	29.05	0.00
56	166.13	0.00	0.00	0.00	64.97	0.00
57	192.98	0.00	*53.17	0.00	50.05	0.00
58	*43.55	0.00	0.00	0.00	25.26	0.00
59	*115.19	0.00	0.00	0.00	34.59	0.00
60	158.04	0.00	0.00	0.00	48.76	0.00
61	207.21	0.00	0.00	0.00	35.31	0.00
62	171.20	0.00	0.00	0.00	46.91	0.00
63	197.18	0.00	0.00	0.00	0.00	0.00
64	173.09	0.00	0.00	0.00	32.97	0.00
65	184.89	0.00	0.00	0.00	87.29	0.00
66	170.36	0.00	0.00	0.00	31.05	0.00

Nest #	ppm Chloride Dec 02 (2)	ppb Nitrite Dec 02 (2)	ppb Bromide Dec 02 (2)	ppm Nitrate Dec 02 (2)	ppm Sulfate Dec 02 (2)
1	71.34	0.00	0.00	9.26	42.90
2	70.86	29.98	0.00	10.47	42.33
3	70.30	35.41	0.00	7.19	41.94
4	*76.40	53.31	0.00	7.89	43.21
5	70.76	9.37	17.85	9.69	42.76
6					
7	71.28	9.74	22.91	8.29	43.17
8	64.81	14.07	21.47	2.60	38.31
9	70.92	0.00	12.67	4.19	41.42
10	70.48	18.23	19.97	9.29	42.93
11	72.90	45.29	8.12	11.05	43.09
12	69.36	21.49	22.14	10.24	41.51
13	70.08	19.37	12.06	7.86	42.47
14	69.42	70.23	6.93	3.62	41.38
15	71.59	87.32	0.00	4.42	*63.58
16	68.93	0.00	8.03	12.44	41.24
17	68.95	*211.28	4.73	5.93	41.36
18	68.22	40.43	11.86	10.12	40.77
19	70.19	0.00	17.67	6.80	41.84
20	69.91	0.00	15.79	12.05	41.82
21	72.06	0.00	17.51	12.17	43.19
22	70.61	0.00	0.00	12.48	42.20
23	69.34	0.00	0.00	12.50	41.56

Nest #	ppm Chloride Dec 02 (2)	ppb Nitrite Dec 02 (2)	ppb Bromide Dec 02 (2)	ppm Nitrate Dec 02 (2)	ppm Sulfate Dec 02 (2)
24	*15.56	36.98	0.00	2.02	*9.47
25	69.73	14.11	0.00	0.02	40.95
26	65.75	*265.95	0.00	7.89	39.52
27	70.27	*418.39	0.00	8.18	42.11
28	65.41	*174.75	0.00	5.89	40.00
29	63.97	0.00	18.01	0.01	*37.49
30	69.59	*257.32	0.00	8.66	41.65
31	67.79	*164.20	0.00	8.64	40.83
32	66.48	19.51	8.91	9.87	40.03
33	67.45	76.03	0.00	10.91	41.53
34	*35.32	0.00	0.00	6.06	*21.34
35	69.00	0.00	0.00	10.37	41.45
36	69.98	0.00	0.00	12.51	41.84
37	69.92	41.41	15.81	5.27	41.85
38	64.54	*116.66	0.00	10.87	38.82
39	66.55	42.50	0.00	10.88	40.10
40	69.78	17.96	0.00	10.71	41.95
41	70.18	52.75	0.00	8.34	43.07
42	70.24	0.00	0.00	12.44	42.20
43	65.92	36.39	0.00	2.87	*38.06
44	68.71	0.00	17.90	12.37	41.30
45	65.62	52.00	0.00	11.10	39.36
46	69.92	58.76	8.69	9.71	42.19
47	69.24	0.00	0.00	12.28	41.47
48	69.03	21.02	0.00	12.26	41.52
49	69.91	11.08	21.57	2.50	41.50
50	68.81	0.00	0.00	12.18	41.01
51	69.81	0.00	0.00	12.43	41.93
52	*56.99	12.19	0.00	7.99	*34.30
53	69.41	0.00	12.63	12.56	41.90
54	71.37	17.46	0.00	11.80	42.61
55	70.55	15.62	0.00	6.03	42.29
56	69.25	0.00	9.56	11.89	41.58
57	70.56	25.86	0.00	11.01	42.32
58	*17.44	0.00	0.00	2.88	*10.97
59	*45.25	31.41	0.00	6.17	*27.30
60	66.00	13.07	0.00	10.32	39.58
61	68.77	0.00	0.00	10.50	41.07
62	67.72	121.62	0.00	10.16	40.79
63	69.96	0.00	0.00	11.85	42.10
64	71.11	0.00	0.00	10.53	42.53
65	70.37	12.69	0.00	11.14	42.42
66	69.68	0.00	7.29	11.85	41.82

Appendix BA: Data Results: Sonde Data, December 2002 – January 2003

Nest #	DateTime	Temp	SpCond	DO Conc	pH	pHmV	ORP
1A	23-Dec-02	9.65	0.92	3.05	7.1	-14.7	-109
2A	23-Dec-02	10.15	0.018	3.53	6.98	-8.1	-106
3A	23-Dec-02	6.27	1.014	1.97	7.2	-20	-121
4A	unavailable	unavailable	unavailable	unavailable	unavailable	unavailable	unavailable
5A	23-Dec-02	5.29	2.124	1.33	6.78	3.2	-100
6A	23-Dec-02	15.59	0.335	5.93	7.09	-14.3	18
1B	23-Dec-02	11.45	1.476	0.84	6.88	-2.4	-108
2B	23-Dec-02	11.17	2.257	1.49	6.73	6.2	-93
3B	23-Dec-02	8.3	0.941	0.48	7.12	-15.6	-103
4B	23-Dec-02	5.03	0.838	0.44	6.88	-2.4	-42
5B	unavailable						
6B	23-Dec-02	9.27	2.21	1.59	6.92	-4.6	-100
1C	23-Dec-02	11.68	0.858	2.88	7.03	-10.9	157
2C	23-Dec-02	12.46	0.852	4.46	7.11	-15.2	-21
3C	23-Dec-02	7.93	0.853	0.46	7.05	-11.8	-51
4C	23-Dec-02	7.87	0.833	0.4	7.11	-15.3	-80
5C	23-Dec-02	11.31	0.84	1.25	7.13	-16.2	-63
6C	23-Dec-02	9.63	0.842	1.24	7.01	-9.6	-6
1A	08-Jan-03	7.98	0.919	2.19	6.99	-8.4	-91
2A	08-Jan-03	8.35	1.791	1.61	6.87	-1.7	-106
3A	08-Jan-03	8.2	1.159	0.89	7.08	-13.3	-120
4A	08-Jan-03	6.27	0.689	11.87	7.13	-16.5	-104
5A	08-Jan-03	6.24	2.265	0.69	6.77	4	-108
6A	08-Jan-03	6.42	1.499	1.78	6.82	1.3	-79
1B	08-Jan-03	9.15	1.128	1.32	6.9	-3.2	-80
2B	08-Jan-03	9.32	2.364	0.89	6.73	6.4	-101
3B	08-Jan-03	10.01	0.952	0.44	7.17	-18.6	-126
4B	08-Jan-03	6.6	0.871	0.42	6.96	-7	-44
5B	08-Jan-03	7.09	1.83	0.56	6.89	-2.8	-94
6B	08-Jan-03	6.92	2.736	0.41	6.8	2	-87
1C	08-Jan-03	11.97	0.871	2.93	7.06	-12.2	0
2C	08-Jan-03	12.16	0.861	4.6	7.11	-15	-34
3C	08-Jan-03	10.47	0.883	0.44	7.14	-17.1	-104
4C	08-Jan-03	10.09	0.869	0.33	7.07	-13.1	-80
5C	08-Jan-03	12.33	0.869	1.44	7.12	-16	-38
6C	08-Jan-03	10.91	0.871	1.61	7.06	-12.5	-34
1A	09-Jan-03	8.33	0.923	0.62	7.07	-13.2	-100
2A	09-Jan-03	8.89	1.79	1.1	6.88	-2.2	-106
3A	09-Jan-03	8.81	1.155	0.44	7.12	-15.8	-118
4A	09-Jan-03	7.04	0.007	5.93	7.24	-22.1	-90
5A	09-Jan-03	6.53	2.257	0.55	6.83	0.3	-109
6A	09-Jan-03	6.83	2.342	1.92	6.81	1.5	-76
1B	09-Jan-03	9.92	1.121	0.52	6.93	-4.8	-90
2B	09-Jan-03	9.88	2.339	0.27	6.79	2.8	-115
3B	09-Jan-03	10.16	0.945	0.1	7.24	-22.5	-118

Nest #	DateTime	Temp	SpCond	DO Conc	pH	pHmV	ORP
4B	09-Jan-03	8.01	0.836	0.15	7.01	-9.7	-22
5B	09-Jan-03	8.58	1.824	0.1	6.95	-6.3	-97
6B	09-Jan-03	7.54	2.347	0.08	6.89	-3	-98
1C	09-Jan-03	11.77	0.872	2	7.06	-12.6	18
2C	09-Jan-03	11.67	0.873	4.07	7.1	-14.6	24
3C	09-Jan-03	10.2	0.865	0.08	7.09	-14.2	-72
4C	09-Jan-03	9.67	0.813	0.13	7.12	-15.9	-63
5C	09-Jan-03	11.57	0.86	1.9	7.12	-15.9	-24
6C	09-Jan-03	10.19	0.849	1.89	7.08	-13.4	2

Appendix BB: Target Analyte Distributions & Statistics

	Strata A			Strata B			Strata C		
Target Analyte	Jan-02	Dec 02 (1)	Dec 02 (2)	Jan-02	Dec 02 (1)	Dec 02 (2)	Jan-02	Dec 02 (1)	Dec 02 (2)
ppb Fluoride	Logistic	Log Logistic	Logistic	Triangular	Inv. Gaussian	Weibull	Weibull	Weibull	Gamma
mean	182.729	201.46	236.804	200.69	276.65	208.736	154.972	162.48	178.0414
mode	182.729	196.06	236.804	192	251.92	189.394	156.331	167.02	175.9095
median	182.729	199.01	236.804	198.64	268.5	203.117	155.367	163.99	177.3317
std.dev.	57.957	36.052	52.213	69.679	75.393	37.723	7.699	13.394	14.1778
variance	3359.041	1299.75	2726.239	4855.11	5684.17	1423.025	59.274	179.39	201.0091
skewness	0	0.6904	0	0.0747	0.6823	0.7495 [est]	-0.2376 [est]	-0.6097 [est]	0.3007
ppb Lactate	Exponential	Uniform	Zero	Lognormal	Triangular	Triangular	LogLogistic	Zero	Zero
mean	1417.8	242.8	0	1433.7	274.64	183.07	16.444	0	0
mode	-1.5968	0	0	534.94	0	0	7.5811	0	0
median	982.22	242.8	0	1069	241.32	160.86	11.521	0	0
std.dev.	1419.3	144.93	0	1388.2	194.2	129.45	34.126	0	0
variance	2014553.9	21005.44	0	1927169.8	37713.58	16757.33	1164.594	0	0
skewness	2	0	N/A	2.5579	0.5657	0.5657	N/A	N/A	N/A
ppb Acetate	Exponential	Zero	Zero	Exponential	Zero	Zero	Lognormal	Zero	Zero
mean	620.67	0	0	435.56	0	0	1.8251	0	0
mode	-7.8068	0	0	-6.8702	0	0	0.0055177	0	0
median	427.82	0	0	299.8	0	0	0.34127	0	0
std.dev.	628.47	0	0	442.43	0	0	9.4613	0	0
variance	394978.2	0	0	195742.97	0	0	89.5163	0	0
skewness	2	N/A	N/A	2	N/A	N/A	153.1895	N/A	N/A
ppb Propionate	Inv. Gaussian	Zero	Zero	BetaGeneral	Zero	Zero	Triangular	Zero	Zero
mean	12.591	0	0	1.1535	0	0	0.46922	0	0
mode	0.0083666	0	0	0	0	0	0	0	0
median	0.27594	0	0	0.18449	0	0	0.41229	0	0
std.dev.	116.649	0	0	1.8467	0	0	0.33179	0	0
variance	13606.946	0	0	3.4103	0	0	0.11008	0	0
skewness	27.7035	N/A	N/A	1.8913	N/A	N/A	0.5657	N/A	N/A
ppb Formate	Normal	Zero	Zero	Triangular	Inv. Gaussian	Logistic	BetaGeneral	Zero	Logistic
mean	6851.8	0	35.495	8657.5	55.348	99.188	245.01	0	48.08
mode	6851.8	0	0	11205	52.535	99.188	N/A	0	48.08
median	6851.8	0	35.495	9130.3	55.348	99.188	34.581	0	48.08
std.dev.	4832	0	21.124	3901.4	37.042	75.972	356.46	0	24.884
variance	23347906	0	446.207	15220931	1372.1	5771.695	127064.3	0	619.191
skewness	0	N/A	0	-0.3639	0.1522	0	1.349	N/A	0
ppb Butyrate	Triangular	Zero	Zero	Zero	Zero	Zero	Zero	Zero	Zero
mean	0.63903	0	0	0	0	0	0	0	0
mode	0	0	0	0	0	0	0	0	0
median	0.56151	0	0	0	0	0	0	0	0
std.dev.	0.45186	0	0	0	0	0	0	0	0
variance	0.20418	0	0	0	0	0	0	0	0
skewness	0.5657	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Target Analyte	Strata A			Strata B			Strata C		
	Jan-02	Dec 02 (1)	Dec 02 (2)	Jan-02	Dec 02 (1)	Dec 02 (2)	Jan-02	Dec 02 (1)	Dec 02 (2)
ppm Chloride	Triangular	Logistic	Logistic	Ext. Value	Weibull	Logistic	Logistic	Logistic	Triangular
mean	71.27	67.9153	73.9253	66.561	60.076	65.4196	81.2113	68.6155	68.9564
mode	77.94	67.9153	73.9253	58.973	62.417	65.4196	81.2113	68.6155	70.24
median	72.574	67.9153	73.9253	63.791	60.828	65.4196	81.2113	68.6155	69.1965
std.dev.	12.324	9.1125	14.9621	16.862	8.0764	7.9229	2.1647	4.1728	2.0179
variance	151.88	83.0368	223.8642	284.326	65.229	62.7724	4.6859	17.4125	4.072
skewness	-0.3089	0	0	1.1395	-0.4805 [est]	0	0	0	-0.3559
ppb Nitrite	Inv. Gaussian	Uniform	Exponential	Exponential	Uniform	Zero	Exponential	Exponential	Triangular
mean	1.5147	12.3	86.939	4.6597	19.74	0	4.4094	92.887	41.501
mode	0.27131	0	-1.3375	-0.066785	N/A	0	-0.065852	-1.5744	0
median	0.91739	12.3	59.851	3.2094	19.74	0	3.0362	63.901	36.466
std.dev.	1.8134	7.3506	88.277	4.7265	11.819	0	4.4753	94.461	29.346
variance	3.2886	54.031	7792.77	22.3397	139.694	0	20.0279	8922.881	861.16
skewness	3.1655	0	2	2	0	N/A	2	2	0.5657
ppb Bromide	Logistic	Beta General	Inv. Gaussian	Triangular	BetaGeneral	BetaGeneral	BetaGeneral	Logistic	Uniform
mean	2.1301	815.22	330.31	2.478	1311.9	1449.6	0.038514	16.5763	11.455
mode	2.1301	N/A	7.153	4.2667	N/A	N/A	0.05	16.5763	N/A
median	2.1301	163.27	92.242	2.695	838.43	1160.9	0.04361	16.5763	11.455
std.dev.	1.53979	1154.9	751.33	1.2648	1305.8	1224.7	0.012678	10.4694	6.8202
variance	2.37095	1333731	564500.28	1.5996	1704984.4	1499950.8	0.0001607	109.6082	46.515
skewness	0	1.3784	6.4789	-0.5657	0.7372	0.5236	-1.1656	0	0
ppm Nitrate	Zero	Pearson	Log-Logistic	Zero	Logistic	LogLogistic	BetaGeneral	BetaGeneral	BetaGeneral
mean	0	0.436697	0.28012	0	0.070507	0.061857	2.8346	8.7698	8.7517
mode	0	0.008478	0.085585	0	0.070507	0.045433	3.79	13.06	12.56
median	0	0.047672	0.16547	0	0.070507	0.054107	3.0267	9.4896	9.7246
std.dev.	0	N/A	N/A	0	0.04033	0.045485	0.78501	3.3339	3.4143
variance	0	N/A	N/A	0	0.0016265	0.0020688	0.61624	11.115	11.658
skewness	N/A	N/A	N/A	0	0	2.7904	-0.8297	-0.7056	-0.7916
ppm Sulfate	Log Logistic	Inv. Gaussian	Lognormal	Inv. Gaussian	Inv. Gaussian	Inv. Gaussian	Logistic	Triangular	Logistic
mean	12.0497	2.1935	13.654	1.9184	6.9186	18.17	49.34761	38.0664	41.69131
mode	3.7361	0.061148	0.13014	0.10841	0.12175	N/A	49.34761	38.22	41.69131
median	6.7665	0.53204	2.4502	0.65974	0.87189	11.746	49.34761	38.1027	41.69131
std.dev.	N/A	5.5409	76.023	3.8237	26.4744	17.761	0.99927	1.2568	1.06099
variance	N/A	30.7019	5779.491	14.6209	700.895	315.464	0.99854	1.5796	1.1257
skewness	N/A	7.3657	191.509	5.7713	11.4164	0.7374	0	-0.0732	0

Appendix BC: Sample Collection Dates (December 2002)

Nest #	A1	A2	B1	B2	C1	C2
1	12/6	12/20	12/9	12/24	12/8	12/30
2	12/6	12/20	12/10	12/24	12/8	12/30
3	12/6	12/20	12/9	12/24	12/8	12/30
4	12/6	12/20	12/10	12/24	12/8	12/30
5	12/7	12/20	12/10	12/24	12/8	12/30
6	12/7	12/20	12/9	12/24	N/A	N/A
7	12/7	12/20	12/9	12/24	12/8	12/30
8	12/7	12/20	12/10	12/24	12/8	12/30
9	12/7	12/20	12/9	12/24	12/8	12/30
10	12/7	12/20	12/10	12/24	12/8	12/30
11	12/7	12/20	12/9	12/31	12/8	12/30
12	12/10	12/20	12/9	12/24	12/8	12/30
13	12/7	12/20	12/10	12/24	12/8	12/30
14	12/18	12/20	12/10	12/24	12/8	12/30
15	12/9	12/20	12/10	12/24	12/8	12/30
16	12/9	12/20	12/10	12/24	12/8	12/30
17	12/7	12/20	12/9	12/25	12/8	12/30
18	12/7	12/21	12/10	12/25	12/8	12/30
19	12/9	12/21	12/9	12/25	12/14	12/30
20	12/9	12/21	12/9	12/25	12/14	12/31
21	12/9	12/21	12/9	12/25	12/14	12/31
22	12/7	12/21	12/9	12/25	12/14	12/31
23	12/9	12/21	12/9	12/25	12/14	12/31
24	12/9	12/21	12/9	12/29	12/14	12/31
25	12/9	12/21	12/9	12/25	12/14	12/31
26	12/8	12/21	12/9	12/25	12/14	12/31
27	12/10	12/21	12/9	12/25	12/14	12/31
28	12/18	12/21	12/9	12/25	12/14	12/31
29	12/9	12/21	12/9	12/25	12/15	12/31
30	12/9	12/21	12/9	12/25	12/15	12/31
31	12/18	12/21	12/9	12/25	12/15	12/31
32	12/18	12/21	12/9	12/25	12/15	12/31
33	12/9	12/21	12/9	12/25	12/15	12/31
34	12/16	12/21	12/9	12/25	12/15	12/31
35	12/16	12/21	12/10	12/25	12/15	12/31
36	12/16	12/21	12/10	12/25	12/15	12/31
37	12/16	12/21	12/11	12/25	12/15	12/31
38	12/16	12/21	12/11	12/29	12/15	12/31
39	12/16	12/21	12/11	12/29	12/15	12/31
40	12/16	12/21	12/11	12/29	12/15	12/31
41	12/16	12/21	12/11	12/29	12/15	12/31
42	12/16	12/21	12/11	12/29	12/15	12/31
43	12/16	12/21	12/11	12/29	12/15	12/31
44	12/16	12/21	12/11	12/29	12/15	12/31

45	12/16	12/21	12/11	12/29	12/15	12/31
46	12/16	12/21	12/11	12/29	12/15	12/31
47	12/16	12/21	12/12	12/29	12/15	12/31
48	12/16	12/21	12/19	12/29	12/15	12/31
49	12/16	12/21	12/11	12/29	12/15	12/31
50	12/16	12/21	12/19	12/29	12/15	12/31
51	12/16	12/21	12/11	12/29	12/15	12/31
52	12/16	12/21	12/12	12/29	12/15	12/31
53	12/16	12/21	12/12	12/29	12/15	12/31
54	12/16	12/22	12/12	12/29	12/15	12/31
55	12/16	12/22	12/11	12/29	12/15	12/31
56	12/16	12/22	12/19	12/29	12/15	12/31
57	12/16	12/22	12/11	12/29	12/15	12/31
58	12/16	12/22	12/11	12/29	12/15	12/31
59	12/16	12/22	12/12	12/29	12/15	12/31
60	12/16	12/22	12/11	12/29	12/15	12/31
61	12/16	12/22	12/11	12/29	12/15	12/31
62	12/16	12/22	12/11	12/29	12/16	12/31
63	12/17	12/22	12/12	12/29	12/16	12/31
64	12/17	12/22	12/12	12/29	12/16	12/31
65	12/17	12/22	12/11	12/29	12/16	12/31
66	12/17	12/22	12/11	12/29	12/16	12/31

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Perchloroethene (PCE), Trichloroethene (TCE) and their degradation products are among the most common organic groundwater contaminants in the United States. Constructed wetlands utilizing upward flow harbor reduction-oxidation conditions that have demonstrated the potential to promote both partial and total mineralization of PCE and TCE through the process of natural attenuation.

Organic acid and inorganic anion concentrations are indicative of reduction-oxidation processes that drive chlorinated ethene degradation. These analytes were investigated to assess their development within three vertically stratified regions of a constructed wetland cell at Wright-Patterson Air Force Base fed by groundwater contaminated with PCE and TCE. Data collected during the months of January 2002, December 2002, and January 2003 revealed changes in the organic acid pool over time and in space that correlated with changes in the inorganic anion pool. Overall organic acid concentrations decreased by an average of 80% over 11 month period, indicating a substantial geochemical evolution of the organic acid pool over this timeframe. Measurements dissolved oxygen and ORP supported the existence of an aerobic region at the base of the wetland, followed by an anaerobic region in the strata above. Significant nitrate and sulfate reduction in the anaerobic region occurred in unison with the emergence of higher concentrations of lactate and formate. Results indicate the reducing conditions and substrates required to support reductive dechlorination of chlorinated ethenes were present in the subsurface of the wetland.

15. SUBJECT TERMS

Constructed Wetlands, Chlorinated Ethenes, Reductive Dechlorination, Organic Acids, Inorganic Anions, Ion Chromatography, Sulfate Reduction, Nitrate Reduction, Fermentation, Formate, Lactate, Acetate

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